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UNIVERSITY OF ALBERTA

# Mechanisms of the Food Effect on Hydralazine Pharmacokinetics

by

Hugh Alexander Semple

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

IN

Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA

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## Dedication

For Merrill, Blair, and our Parents.

## Abstract

Hydralazine is an antihypertensive drug which undergoes extensive first pass metabolism. There are problems with large inter- and intra-individual variations in dose requirements. An interaction with food may contribute to this variability. The objective of this project was to determine the mechanism of the interaction between food and orally administered hydralazine, using the dog as an animal model. An attempt was made to duplicate an assay method wherein hydralazine is derivatized with p-anisaldehyde in blood, but the derivatives were unstable. A new assay was developed, employing p-nitrobenzaldehyde to produce stable derivatives of hydralazine in human and canine blood. The dose-dependency and effect of food on the pharmacokinetics of hydralazine in the dog were studied and comparisons made with published data from humans. The dog may be a good model, having in common with the human high systemic clearance, large volume of distribution, saturation of hepatic metabolism at therapeutic doses and when hydralazine is administered with food, reduced bioavailability. The importance of hepatic blood flow in predicting food-related changes in pharmacokinetics when saturation phenomena are taken into account was examined by computer simulation. Using parameters of propranolol, it was found that changes to $K_m$ and/or $V_{max}$ would dominate in a saturated system, and flow would have little effect on bioavailability. A method was developed to implant catheters in the portal, hepatic and jugular veins and carotid artery, and flow probes on the hepatic artery and portal vein of dogs. This chronic, conscious animal model showed that hydralazine absorption is similar between fasted and fed states but lower than predicted by the AUC method, indicating possible pregastric absorption. Increased hepatic blood flow and intrinsic clearance caused elevated hepatic clearance but systemic metabolism also increased with food. For comparison with the animal experiments and to examine the

interaction between enteral nutrients and hydralazine, a study was performed in humans, in which a standard breakfast or enteral nutrients were given with oral hydralazine. A bolus of enteral nutrients or a standard breakfast but not a 6 h enteral infusion decreased the bioavailability of hydralazine, indicating that rate of nutrient administration, not physical form is important.

## Acknowledgements

The author thanks Dr. Yun Tam and Dr. Ron Coutts for their expert and patient guidance and friendship through a most challenging project. It was a privilege to work under their supervision.

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## Symbols and Abbreviations

| | |
|---|---|
| $\alpha$ | probability of making a type one error |
| ACD | anticoagulant-citrate-dextrose |
| ACS | American Chemical Society |
| ANOVA | analysis of variance |
| AUC | area under the concentration *vs.* time curve |
| $\beta$ | terminal elimination rate constant |
| $C_{liver}$ | concentration of drug in the liver compartment |
| cm | centimeter |
| $C_{max}$ | maximum concentration of drug reached after an oral dose |
| $C_{portal\ vein}$ | concentration of drug in the portal vein |
| Cl | clearance |
| $Cl_{int}$ | hepatic intrinsic clearance |
| $Cl_{oral}$ | apparent oral clearance |
| $Cl_{tb}$ | total body clearance |
| CN | cyano |
| °C | degrees Celsius |
| d | in parentheses means decomposed while melting |
| DNA | deoxyribonucleic acid |
| dpm | disintegrations per minute |
| E | extraction ratio |
| EDTA | disodium ethylenediaminetetraacetate |
| eV | electron volts |
| F | bioavailability |

| | |
|---|---|
| $f_b$ | fraction of unbound drug in the blood |
| g | gram |
| *g* | force of gravity |
| ga | gauge |
| GC | gas chromatograph(y) |
| GI | gastrointestinal (as opposed to PG) |
| h | hour(s) |
| HA | hepatic artery |
| HP | hydralazine (1-hydrazinophthalazine) |
| HPLC | high performance liquid chromatograph(y) |
| HPPA | hydralazine p-anisaldehyde hydrazone |
| HV | hepatic vein |
| id | inside diameter |
| in | inch |
| iv | intravenous |
| $K_a$ | first order absorption rate constant |
| kcal | kilocalorie |
| kg | kilogram |
| $K_m$ | Michaelis constant |
| $K_{mf}$ | pooled Michaelis constant for free drug, equal to the liver concentration of free drug when the velocity of metabolism is one half maximal |
| $K_{mt}$ | pooled Michaelis constant for total drug (free plus bound), equal to the liver concentration of total drug when the velocity of metabolism is one half maximal |
| *l* | liter |
| LC | liquid chromatograph(y) |

| | |
|---|---|
| M | molar |
| mg | milligram |
| MHP | 4-methylhydralazine, used as an internal standard in the assay of HP |
| MHPPA | 4-methylhydralazine p-anisaldehyde hydrazone |
| min | minute(s) |
| µg | microgram |
| µl | microliter |
| ml | milliliter |
| mm | millimeter |
| MPTP | 3-(4-methoxyphenyl)-*s* -triazolo [3,4-*a* ] phthalazine |
| MRT | mean residence time |
| MS | mass spectroscopy |
| MTP | methyltriazolophthalazine |
| m/z | mass to charge ratio |
| N | normal |
| NADPH | reduced nicotinamide adenine dinucleotide |
| ng | nanogram |
| nm | nanometer |
| NMR | nuclear magnetic resonance |
| NPD | nitrogen-phosphorus detection |
| od | outside diameter |
| p | probability of rejecting the null hypothesis when it is true |
| PA | p-anisaldehyde (p-methoxybenzaldehyde) |
| PG | pregastric |
| pg | picogram |
| PLSD | least significant difference |

| | |
|---|---|
| PT | "parallel tube" |
| PTFE | polytetrafluoroethylene |
| PV | portal vein |
| Q | blood flow rate in subscript vessel(s) |
| R | rate of drug leaving the liver via metabolism |
| RBC | red blood cell |
| SD | standard deviation |
| SE | standard error of the mean |
| sec | second(s) |
| $t_{1/2}$ | half life |
| $t_{max}$ | time at which $C_{max}$ is reached |
| TP | triazolophthalazine |
| U | unit(s) |
| U.S.P. | United States Pharmacopeia |
| UV | ultraviolet |
| $V_{liver}$ | volume of distribution of the liver compartment |
| $V_{max}$ | maximum velocity of metabolism |
| $V_S$ | volume of distribution of the systemic compartment |
| $V_{ss}$ | steady-state volume of distribution |
| WS | "well-stirred" |
| $X_a$ | amount of drug dose remaining to be absorbed |
| $X_D$ | the amount of drug in the deep tissue compartment |
| $X_{GI}$ | the amount of drug in the gastrointestinal compartment |
| $X_{GIDR}$ | the amount of drug in the gastrointestinal dose reservoir compartment |
| $X_L$ | the amount of drug in the liver compartment |
| $X_{PG}$ | the amount of drug in the pregastric compartment |

| | |
|---|---|
| $X_{PGDR}$ | the amount of drug in the pregastric dose reservoir compartment |
| $X_S$ | amount of drug in the systemic compartment (ng/kg body weight) |
| $X_T$ | the amount of drug in the tissue compartment |
| $X_{VC}$ | the amount of drug in the vascular compartment |

# 1. Introduction

## 1.1 History of Hydralazine

Hydralazine (1-hydrazinophthalazine, Apresoline®, Ciba Geigy) was introduced into clinical use in 1950 as a peripheral vasodilator to treat hypertension. It decreases peripheral vascular resistance by direct action to relax arteriolar smooth muscle. Hydralazine is only moderately effective when given alone and the large doses required to control hypertension in many patients can lead to drug-induced systemic lupus erythematosis after chronic administration. Hydralazine therefore fell out of favor until the 1970's when it was established that in combination with a diuretic and a β adrenergic blocking drug, much lower doses could be used, reducing the risk of side effects. Setting an appropriate oral dosage regimen remains difficult, however, because of high interindividual variation in hydralazine requirement due in part to polymorphism in a major metabolic pathway, acetylation, but also to other presystemic factors. Due to the success of combination therapy, hydralazine has enjoyed a resurgence which is only now beginning to wane with the advent of the calcium channel blocking drugs to control hypertension.

Hydralazine has also been used in the treatment of congestive heart failure but the doses required are higher than for hypertension. This poses an increased risk to the patient so for this purpose hydralazine is usually prescribed for a short term to stabilize the patient and then the treatment is switched to a safer if less effective drug.

## 1.2 Pharmacology of Hydralazine

Hydralazine has a gradual onset and long duration of action, producing a moderate hypotensive effect. Its mechanism of action has been the subject of some interest during the past decade. It is thought to directly cause relaxation of vascular

smooth muscle. Baker *et al* (1) traced the distribution of radiolabelled hydralazine in rats using whole body autoradiography, and light and electron microscopic autoradiography. Radiolabel was concentrated in the vasculature, especially the elastic laminae and smooth muscle cells, demonstrating the accessibility of drug to the proposed site of action. Jacobs (2) demonstrated that hydralazine inhibited $Ca^{2+}$ dependent ATPase and phosphorylation in bovine carotid artery myofibrils, linking its action, at least in part, to the actomyosin regulatory system. Other mechanisms implicated include mediation by prostaglandins, membrane hyperpolarization and interference with calcium balance in the cell but these have not yet been well defined (3). Importantly, hydralazine seems to produce its effect by a different mechanism from other vasodilators, and it can be effective when other drugs are not (3).

### 1.3 Side Effects of Hydralazine

While acute reactions to hydralazine occur, such as headache, nausea and dizziness, the most important adverse effect is chronic. Less than 10% of patients develop a systemic lupus-like syndrome, usually after more than 2 months of administration, in slow acetylators who are receiving large doses (>200 mg/day) of the drug (4). The mechanism of this toxicity is not yet known, but the parent drug has been implicated in studies of several possible contributory mechanisms at different levels. Hydralazine was shown to affect the B to Z transition of synthetic DNA, indicating that it can alter DNA conformation to a more immunogenic form (5). In addition, hydralazine can bind to DNA through nitrogen-centered free radical formation, increasing both the antigenicity and mutagenicity of the nucleic acid (6). In common with other drugs that induce lupus, hydralazine inhibits complement component C4 (7), and at the cellular level, hydralazine interferes with signal-induced transglutaminase-dependent leucocyte functions essential for immunologic stability (8).

## 1.4 Measurement of Hydralazine in Biological Fluids

Hydralazine has presented a formidable challenge to the analyst, and despite its long tenure in the marketplace, only in the last decade have specific assays been developed. Not only is the drug unstable, with a 10 min $t_{1/2}$ in plasma *in vitro*, but it rapidly forms hydrazones with biogenic ketones and aldehydes in blood, and these hydrazones can interfere with hydralazine determination (9). Prior to 1978 and even afterward, published assays for hydralazine involved derivatization in acidic conditions (10-14). In 1977, however, Reece *et al.* (15) showed that under acidic conditions, the hydrazones were liberated to varying extents to produce "apparent hydralazine" and "real" hydralazine made up only a small portion of this apparent hydralazine. By far, the most abundant constituent of apparent hydralazine was hydralazine pyruvic acid hydrazone.

Reece and Zacest (16) went on to develop a specific but indirect method to measure real hydralazine. Efforts were then directed toward producing an assay in which derivatization was carried out closer to neutral pH, at which hydralazine pyruvic acid hydrazone is more stable. Degen (17) used 2,4-pentanedione to produce a derivative at pH 6.4 but the derivatization time was 40-60 minutes and the yield was low (40%). Reece *et al.* (18) developed a specific plasma assay using nitrite derivatization at pH 5.5. This assay involves a complicated series of rapid sample handling steps to reduce the loss of hydralazine prior to stabilization as the fluorescent tetrazolophthalazine derivative. About the same time, Ludden *et al.* (19) published the first in a series of studies in which plasma hydralazine was reacted at physiological pH with p-anisaldehyde to quickly form a hydrazone derivative. This method was refined to increase its sensitivity to 2 ng/ml (20), then simplified for use in whole blood (21) to reduce the time between sample collection and derivatization. Its advantages over

previously published hydralazine assays are its simplicity and that derivatization is complete less than 10 minutes after a sample is collected, reducing *in vitro* loss of drug. Duplication of the p-anisaldehyde derivative assay was chosen as the first step in this project.

Since the beginning of this project in 1985, two more assays for hydralazine in plasma have been published (22, 23) but neither shows advantages over that of Ludden *et al.* (21) and both have the disadvantage of *in vitro* hydralazine loss during separation of plasma.

## 1.5 Pharmacokinetics of Hydralazine

### 1.5.1 Overview

The clinical pharmacokinetics of hydralazine in humans were reviewed by Ludden *et al.* (9) and more recently with respect to the use of hydralazine in congestive heart failure, by Mulrow and Crawford (24). Where possible, only the results of studies wherein specific assays for hydralazine were used will be reviewed here. A summary of the pharmacokinetic parameters of hydralazine in humans is found in Table 1-1.

### 1.5.2 Absorption

Although the oral absorption of radiolabelled hydralazine has been claimed to be almost complete (25), the extent of unchanged hydralazine absorption cannot be calculated from these data. Intestinal intralumen decomposition and mucosal metabolism could occur before hydralazine has reached the portal circulation. Hydralazine appears to be absorbed quickly after an oral dose. Peak concentrations occur at 10 to 40 min after ingestion of an oral solution (9), and somewhat more slowly after tablets (26).

### 1.5.2.1 Bioavailability

The bioavailability of orally administered hydralazine depends on acetylator phenotype; it is lower in fast acetylators than in slow acetylators. Reece *et al.* reported hydralazine bioavailability for healthy male fast acetylators to be 16 ± 6% and for slow acetylators, 35 ± 4% (26) while the values from a study by Shepherd *et al.* were 10 ± 5% and 31 ± 8%, respectively in older, hypertensive patients (27). They also found no change in bioavailability on repeated oral administration of hydralazine and no accumulation occurred.

The bioavailability of hydralazine is dose-dependent. Shepherd *et al.* examined 9 hypertensive subjects and found that the AUC and $C_{max}$ of hydralazine increased disproportionately between 0.25 and 1.0 mg/kg in slow acetylators and 0.5 and 2.0 mg/kg in fast acetylators (28). They concluded that increased hepatic blood flow and saturation of the gut wall or hepatic metabolism of hydralazine may contribute to the effect. Saturation of the acetylation pathway was implicated.

The effect of release rate of hydralazine from the gastrointestinal tract was recently evaluated by Ludden *et al.* (29). In a study comparing the relative bioavailability of identical doses of immediate and sustained release formulations, rapid release of drug led to higher $C_{max}$ and AUC values. This result would be expected from a drug that exhibited saturation kinetics or from a drug which is incompletely absorbed from sustained release formulations.

All of the above studies use the AUC method to calculate absolute or relative bioavailability, even though saturation of first pass metabolism was implicated. Since it must be assumed that kinetics are linear when the AUC method is used, errors may be introduced into the calculation of bioavailability. This subject requires further study in which more general models are applied.

Food appears to have a significant effect on the bioavailability of hydralazine. This will be discussed in detail in a later section.

### 1.5.3 Distribution

Ludden *et al.* (9) cited several studies in which hydralazine-derived radiolabel was measured in various organs of different species and found to be concentrated in the kidney, liver, blood, adrenals and arteries. Considering the rapid intravascular metabolism rate of hydralazine (about 10 min - see Metabolism section), it is questionable whether any conclusions can be drawn about the distribution of the parent drug from these reports. The study by Baker *et al.* (1) showed concentration of hydralazine radiolabel in the vasculature but again, the chemical species was not identified. Information on the distribution of hydralazine itself is lacking and more study in this area using a specific assay method would be useful.

#### 1.5.3.1 Protein Binding

Lesser *et al.* (25) measured the binding of $^{14}$C-hydralazine in human plasma and albumin (87% and 84%, respectively, using equilibrium dialysis) and the partitioning of hydralazine between buffer and red cells (about .23 dpm/ml in RBC per dpm/ml in buffer). Ludden *et al.* (9) questioned the accuracy of their data, however, due to the instability of hydralazine, especially in plasma. More recently, Ogiso and Iwaki (30) studied the binding of hydralazine and its pyruvate hydrazone to rat plasma protein and human serum albumin by ultrafiltration and equilibrium dialysis. They obtained data in agreement with earlier studies but unfortunately used a non-selective method (12) to measure unchanged hydralazine concentrations. How this affected their results and their stability data is unknown. The binding of hydralazine to rat plasma protein was reported to be 96%, and to human serum albumin 60-80%, with a trend toward decreasing binding with increasing hydralazine concentration. Thermodynamic

analysis and fluorescent probe displacement studies led these authors to conclude that hydralazine is mainly bound to two specific sites for acidic drugs on human serum albumin and that hydralazine may interact with receptor sites on this protein mainly by hydrophobic and hydrogen bonding forces and partly by electrostatic forces.

### 1.5.4 Elimination

After an intravenous dose, hydralazine has a $t_{1/2}$ of about 0.75 h in humans (26, 31). The total body clearance of about 100 ml/min/kg indicates significant extrahepatic metabolism (26, 31). Hydralazine is extensively distributed, with a steady state volume of distribution of more than 1 *l*/kg (26, 31).

#### 1.5.4.1 Metabolism

The metabolism of hydralazine is complex (Figure 1-1). The studies before 1982 were summarized by Ludden *et al.* (9) and an exhaustive review was written in 1977 by Israili and Dayton (32). Three major types of reactions occur, acetylation, oxidation and hydrazone formation. The first two take place in the liver while the last occurs in the vascular compartment. Glucuronidation and sulfation may occur as phase II metabolism.

In humans, acetylation of hydralazine exhibits polymorphism in the population, with slow acetylators mainly excreting oxidation metabolites and fast acetylators excreting more acetylated metabolites (9). The main acetylation metabolite is N-acetylhydrazinophthalazinone but acetylation of hydralazine can also produce a cyclized species, methyltriazolophthalazine (MTP), which undergoes further oxidation to 3-hydroxy-MTP, 3-carboxy-MTP, the aldehyde analogue and then glucuronidation. Hydrolysis of the glucuronide (especially in urine) may result in the formation of triazolophthalazine (TP). Aromatic hydroxylation of hydralazine and MTP can occur at the 9 position. Although most acetylator phenotyping has been carried out using

sulfamethazine, Schmid *et al.* (33) and later, Dubois *et al.* (34) have pointed out that the correlation between hydralazine metabolite concentrations and acetylator phenotype determined from a non-treatment-related foreign compound is poor. A case is presented for the quantification of the main metabolite, N-acetylhydrazinophthalazinone in urine as a means of determining treatment related metabolic status.

Primary oxidation of hydralazine can produce phthalazine, phthalazinone or hydrazinophthalazinone, the last of which is an intermediate in the formation of N-acetylhydrazinophthalazinone. Timbrell *et al.* (35) have questioned whether phthalazinone could be involved in the induction of the lupus syndrome. When examining the metabolite profile differences between slow and fast acetylators, and between slow acetylators with lupus and without the disease, it was found that the sole fast acetylator who had lupus also excreted far more phthalazinone than other fast acetylators. Slow acetylators also produce more of this metabolite. Unfortunately, to date, none of the reported biochemical and immunological studies on hydralazine's interaction with nucleic acids or cells has focussed on phthalazinone. Only the parent drug has been shown to interact with nucleic acids and have other properties consistent with drug induced lupus. Only hydralazine pyruvic acid hydrazone and MHP have been investigated for their lupus-inducing properties; both were found not to have any significant interaction with DNA. More investigation into this subject is required. LaCagnin *et al.* (36) have discovered an unusual oxidative metabolite formed in a rat microsomal preparation, a dimer formed from phthalazine and 1-aminophthalazine. The formation of this species is postulated to involve free radical intermediates. Again, it was suggested that oxidative metabolism on the way to forming this metabolite could be involved in the toxicity of hydralazine, including the lupus syndrome and carcinogenicity.

Hydrazones of hydralazine are formed in blood mainly from pyruvic acid but also in small quantities from other endogenous keto acids and aldehydes. Hydralazine pyruvic acid hydrazone forms rapidly in blood or plasma, with a formation half life of about 10 min, but it is further metabolized to MTP and other products and does not appear in high concentrations in urine. It will be difficult to determine exactly the contribution of each pathway to the metabolism of hydralazine because several pathways converge to form the same metabolites in urine. In addition, only about 55% of the dose is accounted for by the metabolites identified so far, so as yet unidentified metabolites may exist.

### 1.5.5 Interaction Between Hydralazine and Food

The effect of food on the pharmacokinetics of drugs which undergo extensive first pass metabolism has been the subject of interest for over a decade. Melander *et al.* (37), using a non-specific assay method (10), first reported a 2- to 3-fold increase in hydralazine bioavailability when it was administered with food. In a later study, Liedholm *et al.* (38) using a more specific assay method (12), found a much smaller increase in the AUC of "real" hydralazine, and little change to the bioavailability of sustained release hydralazine. Their data was later refuted by that of Shepherd *et al.* (39), who, using an assay for hydralazine which did not employ acidic derivatization conditions, and which they claimed was even more specific than that employed by Liedholm *et al.* (38, 12), found that peak blood hydralazine concentration and AUC were reduced by over 45% after food. Shepherd *et al.* postulated that the increase in pyruvic acid concentrations in the bloodstream after a meal could have led to increased assay interference from hydralazine pyruvic acid hydrazone in the first two studies. There were, however, other differences between the studies, such as subject type, drug formulation, exact meal composition and meal timing which could also have influenced

the results. Indeed, this point was made by Melander *et al.* (40) who remarked that the food effect could have waned by the time the hydralazine was given 45 min later in the study by Shepherd *et al.* In their reply, Shepherd and Ludden (41) presented pharmacodynamic and pharmacokinetic data to support their position. Tachycardia was recorded in both studies after food. Melander *et al.* perceived it to be the result of higher hydralazine concentrations, but Shepherd *et al.* interpreted it as secondary to higher levels of hydralazine pyruvic acid hydrazone, which may cause tachycardia without a hypotensive effect. Unlike the previously mentioned authors, Walden *et al.* (42) reported no effect of food on hydralazine bioavailability. Their data is open to question, however, because the study was flawed in at least two ways. The patients were fed at least an hour before the dose was given and then a non-specific assay was used to measure hydralazine concentrations (10). Shepherd *et al.* (43) repeated their earlier study using conditions similar to those in the Melander study but they again found decreased hydralazine bioavailability when it was given with a meal. In a more recent study, Shepherd *et al.* (44) reported that when a sustained release form of hydralazine was administered with food, the reduction in AUC was less than when regular release drug was given (-29% *vs.* -48%, respectively). Ludden *et al.* (29) observed a decrease in AUC between identical doses of hydralazine solution or regular release tablet given six hours apart. The AUC of the second dose was less than that of the first. The authors speculated that the change could be explained by a more sparse sampling schedule after the second dose, by prior exposure to hydralazine, or by the effect of a lunch given about 4.5 h after the first dose (about 1.5 h prior to the second dose). The concentration of "apparent" hydralazine (which was composed largely of hydralazine pyruvic acid hydrazone) was also measured. Interestingly, the AUC of this species actually rose from the first to the second dose. Ludden *et al.* speculated that increased pyruvic acid levels after a meal may result in increased intravascular metabolism to the

hydrazone, but presented no evidence for their hypothesis. Indeed, pyruvic acid levels are normally in excess of hydralazine concentrations, and the presence of additional pyruvate would be unlikely to significantly affect the rate of hydrazone formation. The increase in pyruvic acid hydrazone is more likely due to an increase in concentration of the limiting substrate, free hydralazine, which could come about through displacement of bound hydralazine from plasma proteins by food constituents. This hypothesis would be difficult to test considering the lability of hydralazine under the conditions necessary to measure protein binding. Despite an apparent resolution of the differences between the original food effect studies, the mechanism of the putative reduction in real hydralazine bioavailability by food remains unknown. The reduction of peak hydralazine concentration may indicate that factors other than increased systemic clearance of hydralazine may be involved.

### 1.5.6 Food Interactions with High First-Pass Drugs

Hydralazine is not the only drug that shows bioavailability changes when administered concomitantly with food, and there is a body of literature written on the effect of food on the bioavailability of drugs which undergo extensive first pass metabolism after an oral dose. Melander *et al.* (45) reported that the bioavailability of propranolol and metoprolol was increased up to two times when they were co-administered with a meal, even though these drugs are almost completely absorbed after an oral dose. Qualitatively similar observations were made on other drugs. McLean *et al.* (46) hypothesized that a transient increase in hepatic blood flow could explain these observations and much of the emphasis of research into the mechanisms of the food effect has focussed on the effect of hepatic blood flow changes on drug bioavailability (47-55). It is now apparent that changes to hepatic blood flow can only partially account for the increases in bioavailability seen, and that other mechanisms must be

sought to fully account for the food effect, including changes to plasma protein and tissue binding, and alterations in the rate of hepatic metabolism. It must be noted that the theory on which the blood flow hypothesis is based carries the assumption that the kinetics of the drugs in question are linear. It is well known, however, that the kinetics of propranolol are nonlinear in the therapeutic range (56-57). The predictions of the model may be significantly altered if the assumption of linearity is relaxed and the theory developed using more general equations. The further development of this theory would aid in the understanding of the food effect on hydralazine kinetics because the oral bioavailability of hydralazine is also dose dependent.

### 1.6 Use of Hydralazine in the Dog

While hypertension is not an important disease of dogs, congestive heart failure is extremely common secondary to mitral valvular endocardiosis in elderly animals. While this problem has been controlled using digitalis glycosides, diuretics and aminophylline, a more general approach was sought earlier in this decade. Hydralazine was one of a number of drugs tested in dogs and found to be useful (58-60). Its effect on hepatic blood flow is similar to that in humans (28, 61-62).

## 1.7 Rationale

The intra- and inter-individual variation in dosage requirements limit the usefulness of hydralazine and create the risk of toxicity. One phenomenon which could contribute to this variability is the effect of food on the bioavailability of orally administered hydralazine. There is disagreement as to whether food increases or decreases bioavailability. The mechanism of the food effect on hydralazine is not known, nor has the mechanism of the food effect on the bioavailability of other drugs subject to extensive first pass metabolism been fully elucidated. The elimination of hydralazine and some other first -pass drugs, for example, propranolol, is dose dependent but the pharmacokinetic theory describing the food effect has not been expanded to include Michaelis-Menten parameters. Because of the problems associated with hydralazine administration and because of the lack of information concerning the mechanism of the food effect on high first pass drugs showing dose-dependent elimination, hydralazine could be a useful model compound.

While the observed problems occur in humans, the pharmacokinetic theory describing the hepatic elimination of drugs uses physiological models. The equations on which these models are based employ input and output concentrations as well as blood flow to the organ of interest, in this case, the liver. These data are not available from humans but can be gathered from an appropriately instrumented animal model. The dog is monogastric like the human, has qualitatively similar gastrointestinal physiology and shows a similar response to hydralazine. It was therefore proposed that the mechanism of the food effect on hydralazine kinetics be studied using the dog as an animal model.

Ultimately, any findings in an animal model must be confirmed or at least supported by data from humans. As well, human data on the food effect could clarify the role of nutrients in changing drug bioavailability.

## 1.8 Hypotheses

1. The bioavailability of orally administered hydralazine is altered by food.
2. The predictions using physiological pharmacokinetic models which describe the food effect on first pass metabolism of high clearance drugs exhibiting saturation in the therapeutic range, will change if Michaelis-Menten equations are used instead of linear equations.
3. The pharmacokinetics of hydralazine in the dog and gastrointestinal physiology of the dog are similar enough to those of the human that the dog would make a useful animal model.
4. A co-administered meal will affect not only hepatic blood flow but also cause transient changes to the rates of metabolism by hepatic enzyme systems. These changes will in turn affect hepatic extraction and clearance during hydralazine absorption.
5. Changes to hepatic metabolism, and not factors affecting absorption, would be the major determinants of bioavailability alterations caused by food.

## 1.9 Objectives

1. To duplicate a published assay or develop a new sensitive, specific method for the measurement of hydralazine concentrations in blood.
2. To evaluate the dog as an animal model of human hydralazine kinetics.
3. To explore the theory surrounding the food effect on first pass metabolism, including the effects of saturation phenomena.
4. To develop a method to instrument dogs in such a way that all the necessary measurements of physiological model parameters can be made in conscious, normal animals; to use these measurements to elucidate the mechanisms by which food may change the bioavailability of hydralazine.
5. To determine the effect of nutrients administered in different forms on the pharmacokinetics of hydralazine in humans and to determine which of the published data can be substantiated.

## 1.10 Table

Table 1-1. Pharmacokinetic parameters reported for hydralazine in humans.

Intravenous dose studies

| Dose (mg/kg) | Plasma (P) or Blood (B) | $Cl_{tb}$ (ml/min/kg) | AUC (ng-min/ml) | $V_{ss}$ (l/kg) | $t_{1/2}$ (min) | β ($min^{-1}$) | Reference number |
|---|---|---|---|---|---|---|---|
| 0.3 | P | 78 | | 1.9 | 54 | 0.013 | 31 |
| 0.38 | P | 138 | 2832 | 6 | 40 | 0.018 | 26 |

Oral dose studies

| Dose (mg/kg) | Acet. phen. | Plasma (P) or Blood (B) | AUC (ng-min/ml) | F (%) | $C_{max}$ (ng/ml) | $t_{max}$ (min) | $t_{1/2}$ (min) | β ($min^{-1}$) | Reference number |
|---|---|---|---|---|---|---|---|---|---|
| 0.25 | slow | B | | | 23 | | | | 28 |
| 0.33 | slow | B | | | 76 | 15 | | | 29 |
| 0.33(tab) | slow | B | | | 49 | 65 | | | 29 |
| 0.5 | slow | P | 1416 | 35 | | | 28 | 0.024 | 26 |
| 0.5 | slow | B | | | 62 | | | | 28 |
| 1 | slow | B | | | 209 | | | | 28 |
| 1 | slow | P | 3680 | 31 | 165 | 18 | 41 | 0.017 | 27 |
| 0.5 | fast | B | | | 15 | | | | 28 |
| 1 | fast | P | 1142 | 16 | | | 26 | 0.026 | 26 |
| 1 | fast | B | | | 65 | | | | 28 |
| 1 | fast | P | 1072 | 10 | 51 | 25 | 13 | 0.053 | 27 |
| 2 | fast | B | | | 302 | | | | 28 |

## 1.11 Figure

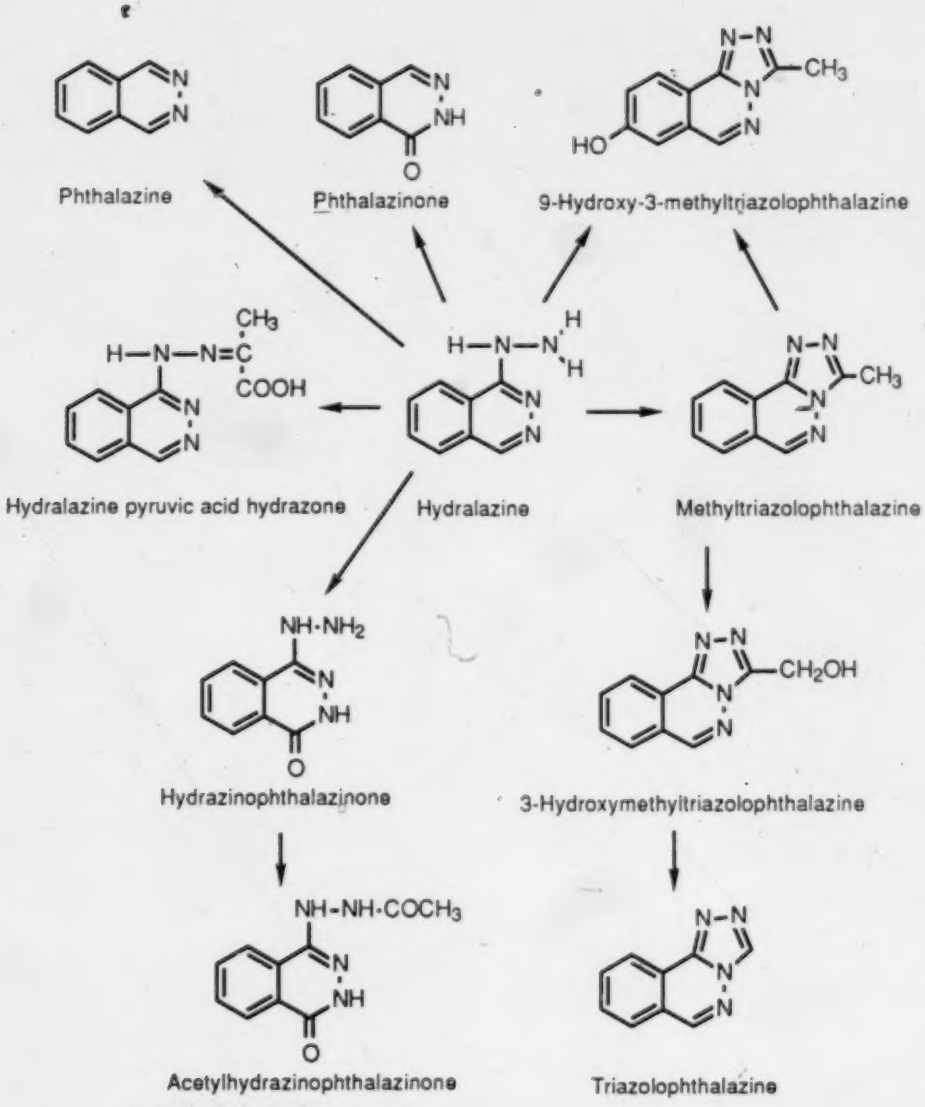


Figure 1-1. Metabolism of Hydralazine

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## 2. Stability Problems With Hydralazine p-Anisaldehyde Hydrazone[1]

### 2.1 Introduction

The quantification of hydralazine (HP) in blood has been a challenging endeavor because its *in vitro* interaction with pyruvic acid to produce the corresponding hydrazone is rapid ($t_{1/2}$≈8-11 min). Ludden *et al*. (1) have shown that the loss of hydralazine in this manner can be arrested by the rapid formation of a derivative, hydralazine p-anisaldehyde hydrazone (HPPA), with p-anisaldehyde (PA) directly in blood. This product can be isolated by a single hexane extraction and measured using HPLC with UV detection. Other advantages of this assay are that it is specific and the procedure is simple. Our studies on the analysis of hydralazine, however, have led us to question the stability of the hydrazone derivatives of HP and its internal standard, 4-methylhydralazine (MHP).

### 2.2 Methods and Results

When we attempted to reproduce the reported (1) assay in canine and human blood, the samples frequently showed significant variability in both HPLC peak area and peak area ratio. Our protocol involved preparing the derivatives in canine blood with EDTA as an anticoagulant or in citrated human blood according to the method described by Ludden *et al*. (1), followed by extraction into 10 ml of hexane. The

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[1] A version of this chapter has been published. H. A. Semple, Y. K. Tam, S. M. Croteau and R. T. Coutts. *J. Pharm. Sci.* 78:432-434 (1989). Reproduced with the permission of the copyright owner, the American Pharmaceutical Association.

supernatant was collected and the solvent removed under calcium chloride-dried nitrogen. The residue contained the desired derivatives and other extracted substances dissolved in about 18 µl of unreacted PA. The samples were stored in this form at -20° C under nitrogen in a borosilicate glass tube tightly sealed with a PTFE-lined cap until the following day when analysis could be performed. Over a one year period, 30 standard curves were prepared and samples in 19 of them gave subnormal responses (leading to poor integration of peaks) and/or highly variable peak height or area ratios. The time span of this problem ranged from losing almost all the samples in a few h to finding only slight variability a day after the samples were prepared.

In an effort to identify possible problems with the samples, stability studies were carried out on fresh canine blood samples containing EDTA as an anticoagulant, human citrated blood samples from a blood bank, and fresh human blood samples containing EDTA. When HPPA was added to whole canine blood with EDTA or to citrated human blood, extracted and immediately chromatographed, the results were erratic. Instability of HP and MHP prior to derivatization, or problems with derivatization or extraction, could not be distinguished from instability of the derivatives when samples were analyzed after storage overnight. Using fresh human blood containing EDTA, which is the condition described by Ludden *et al.* (1), the least variable standard curve was produced when samples were analyzed on the day they were prepared, although the absolute accuracy of the 2 ng/ml samples was poor (Table 2-1). In our hands, however, decomposition of samples led to large changes in slope and poor correlation coefficients after two days (Table 2-1). On day 16 after preparation, the samples had decomposed on average 50%, as is shown by the average internal standard peak area. No clear trends in the data could be observed, however, due in large part to the unpredictable nature of the decomposition process.

Human blood readily available from blood banks for standard curve preparation was citrated. In addition, we wished to adapt the method for canine blood analysis. For these two reasons, we attempted to identify the source of sample variability in these biological fluids. One µl aliquots of the extraction residues were injected undiluted onto a gas chromatograph with a nitrogen-phosphorus detector before dilution of the rest of the sample in HPLC mobile phase and analysis by HPLC. The GC work was performed on a Hewlett Packard Model 5370A instrument equipped with an 18740B capillary control system and an 18789A dual nitrogen-phosphorus/flame ionization detector. A 12 m DB-1 capillary column (J&W Scientific, Folsom, Calif., U.S.A.) was used for separation. The temperature program consisted of a ramp of 16°/min from 100 °C to 280° C, at which temperature the column was flushed for 20 min. The injector and detector temperatures were both set at 250° C and the flow rate of helium carrier gas was 1 ml/min. The LC system consisted of a Model 6000 pump, U6K injector, 20 cm x 4.6 mm µBondapak CN column and Model 440 detector equipped with a 365 nm filter (all from Waters Scientific, Milford, Mass., USA). The mobile phase composition was 70% acetonitrile in acetate buffer (0.9% glacial acetic acid, 0.1% 1 M sodium acetate), pH 3.3, pumped at 2 ml/min.

It was found that samples with aberrant peak areas and area ratios as measured by HPLC also had GC traces different from authentic standards. The standard samples contained two peaks each for HP and MHP. The largest were identified as hydralazine p-anisaldehyde hydrazone (HPPA, Figure 2-1) and its 4-methyl homologue (MHPPA). The smaller two were tentatively identified, using GC/MS, as oxidation products of HPPA, 3-(4-methoxyphenyl)-*s* -triazolo [3,4-*a* ] phthalazine (MPTP, Figure 2-1) and the MHP-derived homologue. GC/NPD and HPLC traces of a blank, a standard sample of HPPA and an aberrant derivatized 300 ng/ml hydralazine sample extracted from canine blood are shown in Figure 2-2. The GC peak at 10.75 min is that of HPPA, and

the one at 11.95 min is MPTP. The mass spectral diagnostic fragments of MPTP are listed in Table II and their structures are included in Figure 2-1. A small amount of MPTP is produced in the sample injector of the GC instrument, and the amount increases with increasing injector temperature. Since PA condenses on the walls of the injection chamber below its boiling point of 248 °C, the injector temperature could not be lowered to entirely eliminate the appearance of an MPTP peak in the standard. Nevertheless, it can be seen that in the sample extracted from blood, much of the derivatized hydralazine appears in the form of MPTP, leading to the conclusion that it was present before injection. The HPLC trace of the same sample reveals the presence of an HPPA peak equivalent to less than 2 ng of HP, a negligible recovery. The presence of MPTP in blood extracts is evidence of oxidative decomposition.

## 2.3 Discussion

A second possible decomposition pathway is hydrolysis of the Schiff's base (HPPA), but direct evidence for this was not obtained. Other putative decomposition peaks also appeared on the chromatograms but these could not be well separated from other early-eluting peaks present in blank and freshly prepared samples. As well, sufficient quantities could not be produced to permit detection and identification by GC/MS. Indirect evidence for hydrolysis of HPPA, however, lies in the greater relative stability of the p-nitrobenzaldehyde hydrazone of hydralazine. This derivative has been found to be quite stable after extraction from canine blood or citrated human blood, and has served as the basis for a new assay (2) which uses similar treatment of the samples to the methods employed here. It is probable that the electronic effect of the aromatic 4-nitro group renders the imino carbon atom less able to accept a weak nucleophile ($OH^-$ or $H_2O$), and therefore the Schiff's base with 4-nitrobenzaldehyde would be more resistant to hydrolysis than the Schiff's base with p-anisaldehyde. Why decomposition

of HPPA would be more prevalent in samples extracted from canine or citrated human blood, and why some samples in a run are affected more than others, is unknown.

We conclude that HPPA and MHPPA are erratically unstable under the described conditions. The instability may lead to sample decomposition, and hence variability and possible errors in estimation of HP concentration. p-Nitrobenzaldehyde can be used to produce a more stable derivative with otherwise similar characteristics to HPPA. For this reason, we recommend the assay for hydralazine in blood developed using p-nitrobenzaldehyde as a derivatizing agent (2).

## 2.4 Tables

Table 2-1. Concentration data from a 16 day stability study on p-anisaldehyde-derivatized hydralazine prepared in fresh human blood containing EDTA.

| | CONCENTRATION (ng/ml) | | | |
|---|---|---|---|---|
| ADDED | DAY 0* | DAY 2* | DAY 8* | DAY 16* |
| 2 | 4.3 | 1.8 | 1.9 | 2.8 |
| 2 | 4.2 | 2.3 | Decomposed | 1.6 |
| 2 | 3.1 | 2.9 | 2.9 | 2.8 |
| 2 | 3.5 | 2.0 | 2.1 | 2.9 |
| 20 | 13.9 | 10.3 | Decomposed | 14.4 |
| 20 | 22.6 | 8.5 | 9.6 | 11.0 |
| 20 | 21.4 | 7.3 | 11.7 | 14.9 |
| 20 | 14.9 | 10.0 | 12.1 | 12.0 |
| 200 | 201.7 | 59.2 | 108.8 | 256.9 |
| 200 | 146.8 | 66.8 | 245.9 | 360.1 |
| 200 | 203.1 | 111.5 | 180.9 | 235.3 |
| 200 | 249.1 | 77.7 | 104.9 | 244.7 |
| Int. Std. Average Peak Area as % of Day 0 | 100.0 | 78.2 | 81.4 | 50.8 |
| Slope^ | 0.159 | 0.062 | 0.129 | 0.223 |
| Intercept^ | 0.014 | 0.218 | -0.330 | -1.161 |
| Coefficient of Determination^ | 0.947 | 0.899 | 0.807 | 0.947 |

* Concentrations were calculated using the slope derived from Day 0 data.

^Slope, intercept and coefficient of determination were calculated from each day's data.

Table 2-2. Fragmentation of peak at 11.95 min.[1]

| m/z | Structure | % Relative Abundance |
|---|---|---|
| 276 | $M^{+\cdot}$ | 100 |
| 275 | $[M-H]^+$ | 49 |
| 261 | $[M-CH_3]^+$ | 16 |
| 233 | $[261-CO]^+$ | 19 |

[1]The instrumentation consisted of a Hewlett Packard Model 5710A gas chromatograph coupled to a Model 5981A mass spectrometer and a Model 5934A 21MX data system. The ionization voltage was 70 eV. The GC conditions were as described for the GC/NPD instrument.

## 2.5 Figures

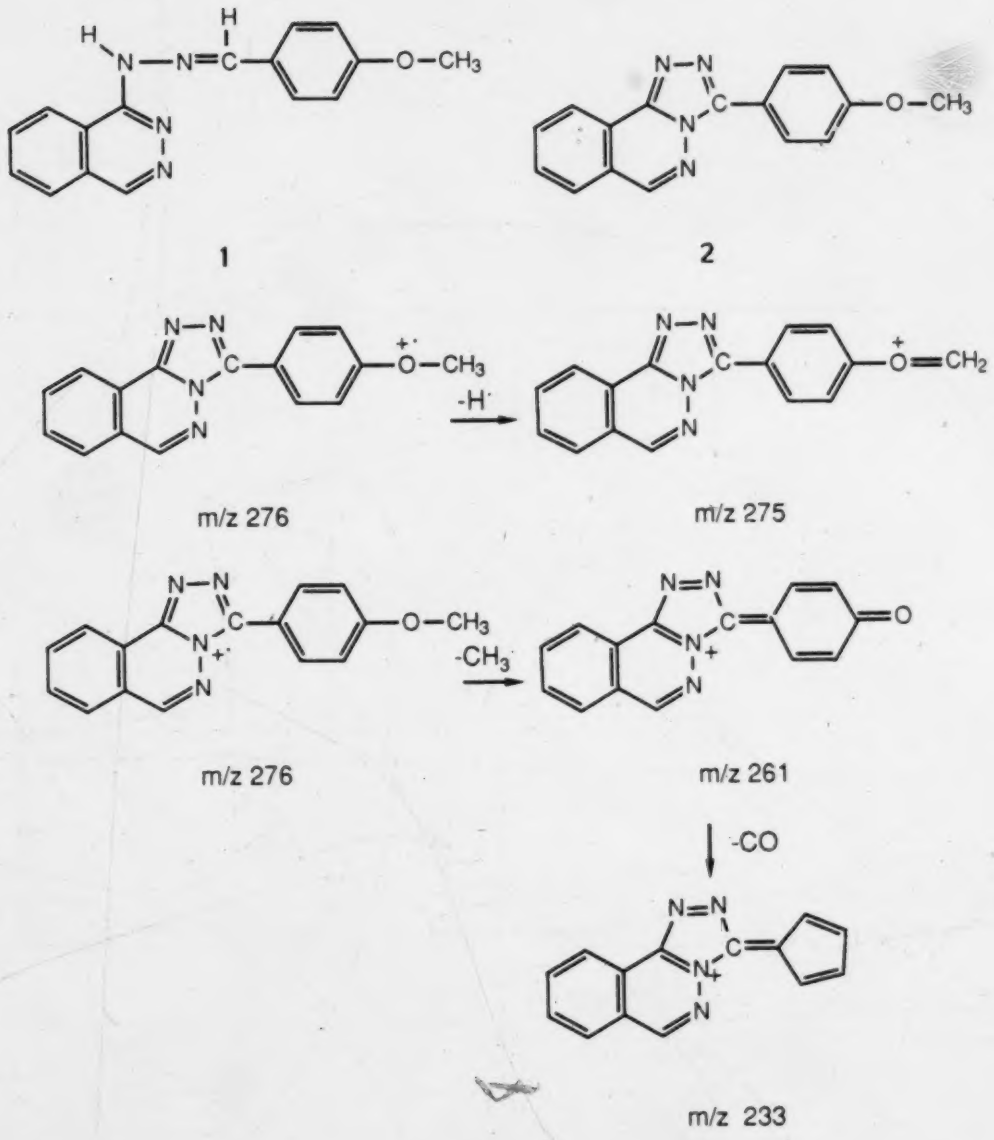


Figure 2-1. Structures of HPPA (1) and MPTP (2) with suggested structures for MPTP mass spectral fragment ions.

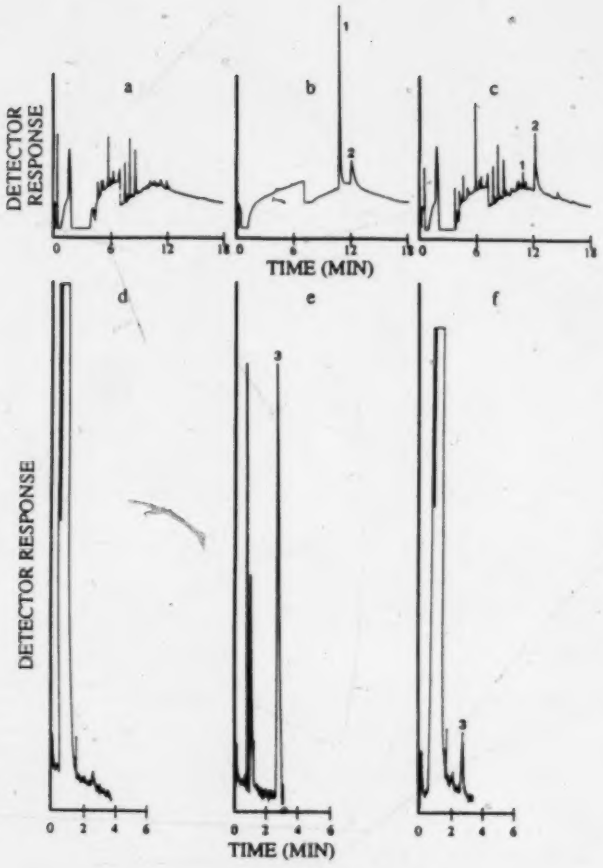


Figure 2-2. GC-NPD traces of a canine blood blank with PA (a), HPPA standard dissolved in ethyl acetate (b), a derivatized hydralazine sample extracted from spiked canine blood, after one day's storage (c), and HPLC traces of the same samples (d,e,f). The GC peak at 10.75 min (1) corresponds to HPPA and at 11.95 min to MPTP (2). The LC peak at 2.65 min corresponds to HPPA (3); MPTP is apparently not separated with this system.

## 2.6 References

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## 3. Assay For Hydralazine as its Stable p-Nitrobenzaldehyde Hydrazone[1]

### 3.1 Introduction

Hydralazine is a peripheral vasodilator which has been widely used in the treatment of hypertension during the last thirty years. Pharmacokinetic studies have been hampered because many early assays lacked specificity, and because the drug is very labile in plasma (1). Several recent specific HPLC assays for hydralazine in plasma have been published (2-6). All of these are complicated by requiring procedures for rapid cooling and/or separation of plasma immediately after withdrawing each blood sample. Ludden *et al.* have used a simpler method, in which a Schiff's base between hydralazine and p-anisaldehyde is formed directly in blood (7). The hydralazine derivative is easily purified with a single hexane extraction prior to HPLC analysis. The assay is selective because the reaction is carried out at physiological pH, and hydrolysis of interfering endogenous hydrazone metabolites is minimal. The hydralazine p-anisaldehyde hydrazone derivative utilized in this assay has, however, been found to be erratically unstable on storage, even overnight at -20° C (8). A replacement for p-anisaldehyde was sought, which would retain the selectivity and sensitivity of the assay but produce a more stable derivative. p-Nitrobenzaldehyde, which contains a strongly electron-withdrawing substituent, was used to produce a more stable Schiff's base. This report describes an HPLC assay of hydralazine as its p-nitrobenzaldehyde

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[1] A version of this chapter has been published. H. A. Semple, Y. K. Tam, S. Tin, and R. T. Coutts. *Pharm. Res.* 5: 383-386 (1988).

hydrazone, which can be stored in extracted samples for prolonged periods without decomposition.

## 3.2 Materials and Methods

### 3.2.1 Reagents and Chemicals

Acetonitrile and hexane were HPLC grade solvents (Fisher Scientific Co., Fairlawn, NJ). Methylene chloride was ACS grade (Anachemia, Montreal, PQ). Hydralazine hydrochloride (Sigma Chemical Co., St. Louis, MO), 4-methylhydralazine, hydralazine pyruvic acid hydrazone (Ciba-Geigy Co., Basle, Switzerland), triethylamine, phosphoric acid, 85% (Fisher Scientific Co., Fairlawn, NJ), p-nitrobenzaldehyde and deuterochloroform (Aldrich Chemical Co., Milwaukee, WI) were used as received. (1,4)-Dioxane was glass distilled before use.

### 3.2.2 Preparation of Hydralazine p-Nitrobenzaldehyde Hydrazone

To 150 ml methylene chloride were added 0.75 g p-nitrobenzaldehyde and 1 g hydralazine hydrochloride to give approximately equimolar quantities. Triethylamine (0.5 ml) was added to neutralize the hydrochloride and liberate the hydralazine free base. A drying tube was attached and the mixture stirred overnight. The resulting orange solution was filtered and evaporated to dryness. The residue was dissolved in a minimum volume of warmed methylene chloride and cooled until recrystallized. The solution was removed by filtration and the crystals dried under vacuum at 80° C. The identity and purity of the product were verified using GC/MS, UV, NMR, and melting point determination.

### 3.2.3 Glassware Treatment

Glassware was washed in FL-70 (Fisher Scientific Co., Fairlawn, NJ), rinsed in deionized, distilled water and muffled at 500° C for 2 h, except for volumetric flasks which were cleaned with chromic acid.

### 3.2.4 Standard Curves

All standard solutions were prepared daily. Hydralazine HCl (61.4 mg) was dissolved in 0.01 N HCl to make 100 ml of stock solution containing 0.5 mg/ml of free hydralazine base. From dilutions of the stock solution, three standard solutions were prepared containing 5 ng/µl, 500 and 100 pg/µl in 0.01 N HCl. The internal standard solution was prepared by dissolving 100 µg of 4-methylhydralazine in 50 ml of 0.01 N HCl. The derivatizing agent was prepared by dissolving 100 mg of p-nitrobenzaldehyde in 1 ml of dioxane.

Blood samples were collected into 60 ml plastic syringes, using EDTA as an anticoagulant. Aliquots of 1 or 3 ml were dispensed into 16x150 mm borosilicate glass tubes. Derivatizing agent (20 µl), internal standard (25 µl) and an appropriate amount of hydralazine were added to each blood sample. The tubes were capped with PTFE-lined caps, vortexed vigorously for 15 sec and allowed to stand for 10 min. The derivatives were extracted by adding 3 volumes of hexane to the tubes and mixing the samples on a Labquake® rotary mixer for 10 min. The samples were centrifuged at 1000 x *g* for 10 min and the supernatants transferred to 16x100 mm glass tubes. The hexane was evaporated under a gentle stream of nitrogen and the extracted samples stored capped at -20° C until analysis.

### 3.2.5 Chromatographic Conditions

Each extraction residue was dissolved in 150 µl mobile phase and the entire sample injected onto a 150x4.6 mm id Ultremex® 3CN (Phenomenex, Palos Verdes, CA) reversed phase column. The aqueous portion of the mobile phase contained 0.1% phosphoric acid (v/v) and 0.1% triethylamine (v/v) in deionized, double distilled water, pH 3.0. The mobile phase consisted of 63% acetonitrile in aqueous phase, pumped at a flow rate of 2 ml/min. Detection was at 365 nm. The HPLC instrumentation included a Model 6000 pump, U6K injector and Model 440 UV absorbance detector equipped with a 365 nm filter (all from Waters & Associates, Milford, Mass., U.S.A.).

### 3.2.6 Animal and Human Studies

The application of this assay to both human and canine subjects was demonstrated by measuring hydralazine concentrations after giving a 50 mg oral dose of Apresoline 20 mg/ml solution (2.5 ml), followed by 100 ml of $H_2O$ to an 86 kg human volunteer and after dosing a 30 kg canine subject iv with 1 mg/kg Apresoline solution (1.5 ml). Blood samples (1 or 3 ml) were collected from the subjects into 3 ml plastic syringes whose hubs were filled with 0.1 M EDTA (approximately 0.05 ml). The syringes were quickly filled, then immediately emptied into 16x100 mm glass tubes. The internal standard and derivatizing reagent were added and the tube vortexed. The time from sampling to vortexing was about 30 seconds. The samples were then treated as described for the standard samples.

### 3.2.7 Data Analysis

The standard curve was evaluated by linear regression analysis of peak area ratio *vs.* concentration. The data were expressed as mean +/- SD (n = 5). The two-tailed

Student's t-test was used to test for differences between groups ($p = 0.05$). The data were fitted using LAGRAN (9).

## 3.3 Results

### 3.3.1 Structure Confirmation for Hydralazine p-Nitrobenzaldehyde Hydrazone

The chemical identity and purity of the p-nitro derivative was confirmed by GC/MS and melting point determination, UV and NMR spectrometry. The major diagnostic MS fragments are summarized in Table 3-1. The melting point of 234-236° C (d), the UV spectrum in mobile phase with a wavelength of maximum absorption of 356.5 nm, and the proton NMR spectrum of hydralazine p-nitrobenzaldehyde hydrazone measured in deuterochloroform were in good agreement with previously published data (10). A trace amount of contaminating hydrazine detected with NMR did not appear to affect the HPLC procedures.

### 3.3.2 Chromatography

The retention time of hydralazine p-nitrobenzaldehyde hydrazone was 3.76 min and that of 4-methylhydralazine p-nitrobenzaldehyde hydrazone, 4.88 min (Figure 3-1). Reagent blanks and reagent blanks containing internal standard showed no interfering peaks. In dog blood, the standard curve was linear over the range 300 pg/ml to 640 ng/ml with a coefficient of correlation of 0.992 for 3 ml samples (Table 3-2). The mean relative standard deviation was 5.8% (range 2.55%-11.97%) with no loss of precision at lower concentrations. Human data were similar, with a correlation coefficient of 0.999. For 1 ml and 3 ml blood samples, the sensitivity was better than 1 ng/ml and 300 pg/ml, respectively. The detection limit (when peak height reached three times the baseline thickness) was 33 pg/ml for 3 ml blood samples. Over 90% of the

derivatization occurred within 3 min; the reaction was virtually complete after 5 min. The derivatization and extraction efficiencies were 96 +/- 4.0% and 74 +/- 2.8%, respectively for a total recovery of 71 +/- 1.9%.

Ludden *et al.* (7) have noted that hydralazine pyruvic acid hydrazone is the only circulating metabolite likely to interfere significantly with their assay by hydrolyzing during the derivatization process in whole blood, to liberate free derivatizable hydralazine. They found that less than 0.05 moles % of hydralazine pyruvic acid hydrazone was detected as hydralazine using whole blood derivatization. Because the new derivative was also formed in whole blood, the selectivity of the new assay should be the same. The two assays were therefore compared for hydralazine production from hydralazine pyruvic acid hydrazone. When p-nitrobenzaldehyde and p-anisaldehyde were separately added to canine blood samples containing 1 μg of hydralazine pyruvic acid hydrazone, there was no significant difference between the two methods in the amount of hydralazine detected (0.30 *vs.* 0.35 moles %, respectively). This suggests that the selectivity of the two procedures is the same and depends on the derivatization conditions rather than the derivatizing agent. The amount of hydralazine detected in our experiment was higher because we used canine blood, in which the stability of hydralazine pyruvic acid hydrazone is less. None of the metabolites, 3-methyl-s-triazolo-[3,4-a]-phthalazine, s-triazolo-[3,4-a]-phthalazine, 4-hydrazino-phthalazin-1-one and 4-(2-acetylhydrazino)phthalazine and phthalazin-1-one produced peaks on the chromatograms.

Standard curves from freshly extracted samples and extracted samples stored for seven days at room temperature or -20° C were compared. Both fresh, whole canine and citrated human blood were evaluated. The slopes from canine samples +/- SD (expressed as a percentage of the slope) were 0.0083 +/- 7.1% for fresh, 0.0088 +/- 7.2% for room temperature stored and 0.0089 +/- 10% for -20° C stored samples.

From human samples, the slopes were 0.0103 +/- 11.7 % for fresh, 0.0099 +/- 10.5% for room temperature stored and 0.0096 +/- 9.7% for -20° C stored samples. The slopes obtained from the different conditions did not differ from each other significantly.

Figures 3-2 and 3-3 show blood concentration *vs.* time data collected from the human and canine subjects. The curve for the human subject had a peak concentration of 7 ng/ml and a terminal half life of 70 min. The dog concentration *vs.* time data showed a biexponential decline with a terminal half life of 151 min.

### 3.4 Discussion

The advantages of the method of Ludden *et al.* are retained by the new hydralazine assay. It is selective and the procedure is simple when compared with recently published assays for hydralazine which require plasma separation plus one or more derivatization and extraction steps (2-6). It is superior to the p-anisaldehyde method because the hydralazine p-nitrobenzaldehyde hydrazone derivative provides an increase in sample stability, allowing prolonged storage of samples before analysis. The blood profiles of hydralazine in the dog using the two assays are shown in Figure 3. Several aberrations can be seen in the curve produced from the p-anisaldehyde assay (Figure 3-3B) and in our hands, this profile could not be reproduced in the same dog on different occasions due to the instability of p-anisaldehyde hydrazone.

The new assay is three times more sensitive than the p-anisaldehyde assay, so is comparable to the more involved plasma assays using electrochemical detection (5,6). With these two improvements, the blood profile after an oral dose can now be better characterized in humans. If a kinetic analysis were to be performed on the human curve (Figure 3-2), using only the data above 1 ng/ml, the $t_{1/2}$ would be 30.5 min, which is comparable to what has already been reported (11,12). Using the new method,

however, a slower decline phase was discovered. This observation is consistent with the hypothesis of Ludden *et al.*, that the systemic elimination of hydralazine is limited by its return to the central compartment or by back conversion of labile metabolites (13). More studies are required to confirm this information.

The data presented here demonstrate the stability of the p-nitrobenzaldehyde hydrazones of hydralazine and 4-methylhydralazine on storage. This method provides a sensitive, selective and simple procedure for the determination of hydralazine concentrations in whole blood, and will be used to study the mechanism of hepatic extraction of hydralazine in the dog and human.

## 3.5 Tables

Table 3-1. Diagnostic fragments from reaction product of hydralazine HCl and p-nitrobenzaldehyde.[1]

| m/z | Relative Abundance(%) | Structure |
|---|---|---|
| 293 | 33 | $M^{+\cdot}$ |
| 292 | 12.4 | $[M-H]^+$ |
| 171 | 100 | $[M-PhNO_2]^+$ |

[1]The instrumentation consisted of a Hewlett Packard Model 5710A gas chromatograph coupled to a Model 5981A mass spectrometer and a Model 5934A 21MX data system. The ionization voltage was 70 eV. A 30 m DB-1 column (J&W Scientific, Folsom, Calif., U.S.A.) was used for separation. The temperature program consisted of 4 min at 100° C, followed by a ramp of 16°/min to 280°, at which temperature the column was flushed for 16 min. The injector and detector temperatures were both set at 250° C and the flow rate of helium carrier gas was 1 ml/min. The retention time of the reaction product peak was 18.6 min.

Table 3-2. Hydralazine standard curve accuracy and precision

| Concentration Added (ng/ml) | Concentration Calculated (ng/ml) | Precision ± 1 SD in % |
|---|---|---|
| 0.3 | 0.29 | 4.76 |
| 1 | 0.89 | 11.97 |
| 2 | 1.74 | 3.00 |
| 8 | 7.50 | 2.55 |
| 40 | 41 | 3.27 |
| 160 | 161 | 5.96 |
| 640 | 650 | 9.11 |

## 3.6 Figures

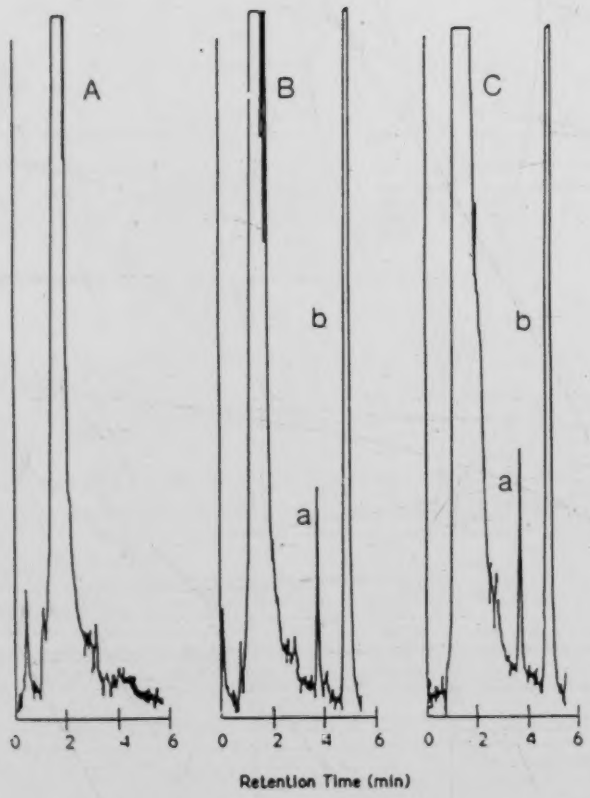


Figure 3-1. Typical HPLC traces of p-nitrobenzaldehyde hydrazones of hydralazine (retention time = 3.76 min, a) and internal standard, 4-methylhydralazine (retention time = 4.88 min, b). A. Reagent blank in blood; B. Spiked 1 ml blood sample from standard curve, 2 ng/ml; C. 1 ml blood sample from dog 480 min after a 1 mg/kg iv dose of hydralazine HCl.

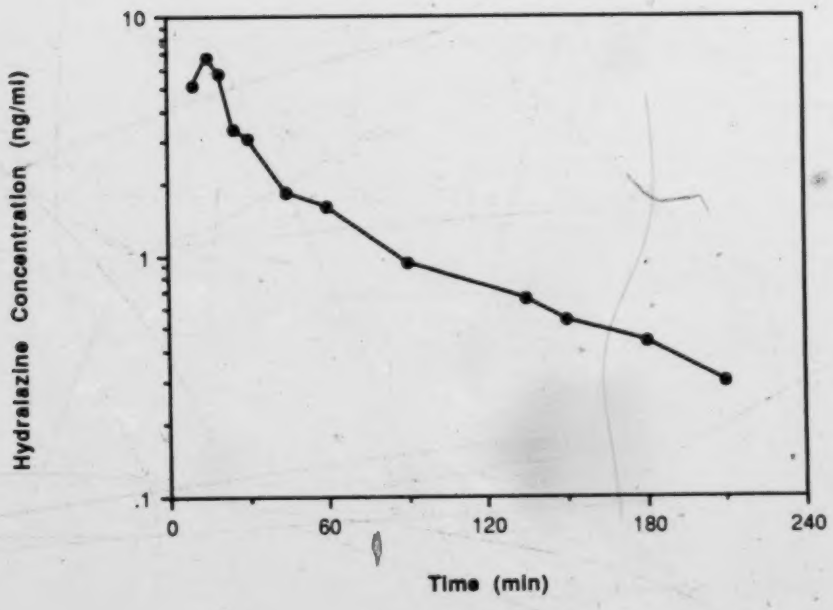


Figure 3-2. Blood concentration *vs.* time curve for an 86 kg human subject given a 50 mg oral dose of hydralazine.

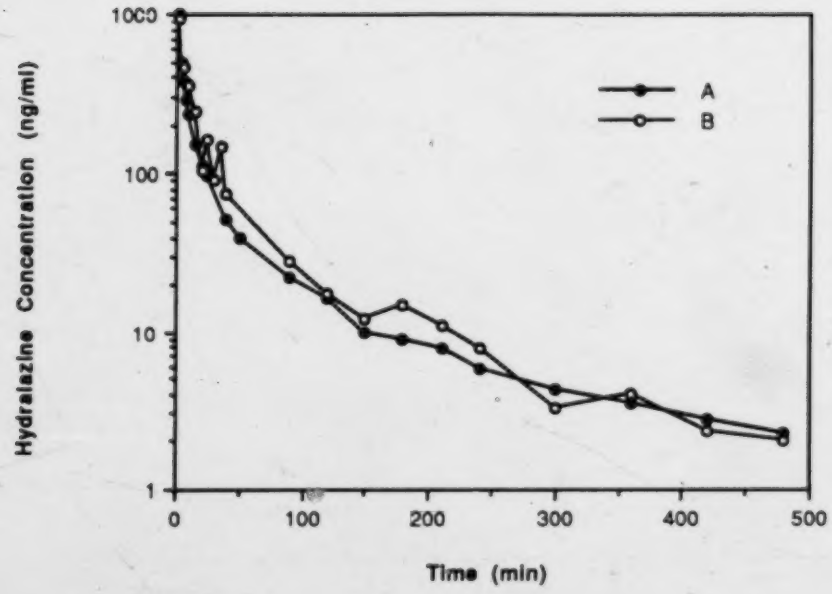


Figure 3-3. Blood concentration *vs.* time curves for a dog given a 1 mg/kg intravenous dose of hydralazine. A. Using p-nitrobenzaldehyde as derivatizing agent and assay as described in the text. B. Using p-anisaldehyde as derivatizing agent and assay by the method of Ludden *et al.* (7).

## 3.7 References


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# 4. A Computer Simulation of the Food Effect: Transient Changes in Hepatic Blood Flow and Michaelis-Menten Parameters as Mediators of Hepatic First Pass Metabolism and Bioavailability of Propranolol[1]

## 4.1 Introduction

The effect of food ingestion on the bioavailability of drugs subject to extensive hepatic first pass metabolism, of which propranolol is the best studied example, can be profound. When propranolol is administered orally along with a meal, an increase in area under the concentration-time curve as large as 100% or more is not uncommon (1-3). It was believed that transient changes in hepatic blood flow in response to a meal could account for nearly all of the increase in the AUC of propranolol (3-6), but it is becoming apparent that mechanisms involving changes in hepatic blood flow ($Q_h$), plasma protein ($f_b$) and hepatic tissue binding, and gut and hepatic metabolism ($K_{mf}$, $V_{max}$) may all contribute to the food effect (7-9). While some of the parameter changes contributing to the food effect, such as hepatic blood flow and plasma protein binding can be measured, investigators have had great difficulty in determining their relative contributions to the observed increases in AUC, due to variability in the data, to the indirect means used to make the measurements, and the different models used to make the predictions (7).

Some insight has been gained from computer simulations, but the conclusions reached have depended on the assumptions made to simplify the model (4, 10, 11, 12).

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[1] A version of this chapter has been accepted for publication. H. A. Semple, Y. K. Tam and R. T. Coutts. *Biopharm. Drug Disp.* (1990)

It is apparent that a more realistic model could help to elucidate the sensitivity of the system to changes in each physiological parameter which contributes to bioavailability. In addition, the effects of the duration of parameter change and of input rate would be of interest, because different drugs and/or formulations are absorbed at different rates relative to the onset and cessation of the food effect. Of particular value would be the inclusion of Michaelis-Menten parameters in the model. Previous models have assumed linear kinetics although the elimination of propranolol is saturable after oral dosing (7, 12). Wagner (13) has published pooled Michaelis-Menten parameters for propranolol based on steady state oral data. Although these parameters do not predict the linear dose-peak concentration relationship seen at higher doses (14), the non-linear portion of the curve seen at doses below 400 mg/day is fitted well. We were interested in modelling single doses of 20, 40 and 80 mg (corresponding to 80, 160 and 320 mg/day), which are frequently used in pharmacokinetic studies of propranolol, 80 mg being the standard single dose used in food effect experiments. Therefore, the pooled parameters were considered to be adequate for our purposes. The linear portion of the dose *vs*. peak concentration curve may be explained by the existence of low-affinity linear pathways which achieve importance only at higher dosage levels where the $V_{max}$ of the saturable pathways is exceeded.

This study introduces Michaelis-Menten kinetics into food effect modelling and utilizes transient changes in the three variable parameters ($Q_h$, $K_{mt}$ and $V_{max}$) to partially mimic the transient effect of a meal consumed with the drug dose. In order to understand the possible role of each parameter in the pharmacokinetic expression of the food effect, a computer simulation model was constructed based on pharmacokinetic data gathered from human and canine studies of propranolol disposition and on physiologic data relevant to the food effect.

## 4.2 Methods

### 4.2.1 Theoretical

A three compartment mass transfer model was constructed on a physiological basis (see Figure 4-1). An oral dose, $X_a$, was absorbed from the gut compartment into the mesenteric circulation which emptied into the liver compartment. The mesenteric circulation was assumed to carry the entire blood supply to the liver. The liver compartment eliminated drug via metabolism and the hepatic vein which emptied into the systemic compartment. All elimination from the model was assumed to be via metabolism from the liver compartment. The systemic compartment in turn received drug from the liver and drained into the mesenteric circulation to complete a loop. The Venous Equilibrium Model (15) was used to describe the liver compartment. There is evidence in the literature that this model may be valid for propranolol (16, 17, 18). In addition, it has been demonstrated that there is poor discrimination between the Venous Equilibrium and other models, such as the Sinusoidal Perfusion Model, at extraction ratios of less than 0.9. The extraction ratio is 0.85 or less throughout the simulations, and during the absorption phase, when first pass metabolism occurs, can drop to less than 0.5. Therefore, the Sinusoidal Perfusion model would be expected to behave similarly to the Venous Equilibrium model and the conclusions would not be changed. Therefore, the simulations were not repeated using this model.

### 4.2.2 Equations

The mass balance for the liver compartment was described by the following differential equation, based on the Venous Equilibrium Model (15):

Rate of change of concentration in the liver,

$$\frac{dC_{liver}}{dt} = \frac{(C_{portal\ vein} - C_{liver}) * Q_h}{V_{liver}} - R \quad (1)$$

Rate of hepatic metabolism,

$$R = \frac{V_{max} * C_{liver}}{(K_{mt} + C_{liver}) V_{liver}} \quad (2)$$

The equations describing the flow of drug through the model are as follows:

Rate of delivery from dose in gut,

$$\frac{dX_a}{dt} = -K_a * X_a \quad (3)$$

Concentration of drug in the portal vein,

$$C_{portal\ vein} = X_S/V_S + K_a * X_a/Q_h \quad (4)$$

Rate of change of amount of drug in the systemic compartment,

$$\frac{dX_S}{dt} = (C_{liver} - X_S/V_S) * Q_h \quad (5)$$

Hepatic extraction ratio,

$$E = (C_{portal\ vein} - C_{liver})/C_{portal\ vein} \quad (6)$$

Hepatic clearance,

$$Cl = E * Q_h \quad (7)$$

Area under the concentration *vs.* time curve

$$AUC = \int_0^\infty X_S\, dt \quad (8)$$

Numerical integration was performed on an Apple MacIntosh computer with the modelling program, Stella (High Performance Systems, Lyme, NH), using the fourth

order Runge-Kutta method with a step size of 1 min and simulation duration of 1440 min when $K_a = 0.01$ min$^{-1}$ and 2000 min when $K_a = 0.002$ min$^{-1}$.

The average pooled Michaelis-Menten parameters for propranolol, as calculated from steady state data by Wagner (13), were expressed on a per kg body weight basis, converted for use in the liver compartment, and inserted into the mass balance equation. The standard set of conditions against which variations were compared comprised the "Basic Model":

$X_a$= 1 mg/kg

$K_a = 0.01$ min$^{-1}$

$V_{max}$ = 5000 ng/min/kg (13)

$K_{mt}$ = 50 ng/ml (13)

$Q_h$ = 20 ml/kg/min (19)

$V_{liver}$ = 80 ml/kg (a value of 20 ml/kg was calculated from the standard of 1.5 *l*/70 kg man (19) and multiplied by 4 to account for the volume of distribution of propranolol which is about 4 *l*/kg. This assumes that the liver has the same relative volume of distribution as the other tissues.)

$V_S$ = 4 *l*/kg

The three parameters which could change in response to food are $Q_h$, $V_{max}$ and $K_{mt}$. $Q_h$ and $V_{max}$ are independent, but it should be noted that $K_{mt} = K_{mf}/f_b$. Decreasing $f_b$ or increasing $K_{mf}$ by the same factor would have identical effects on $K_{mt}$. These two parameters can be distinguished experimentally, but not mathematically, so $f_b$ and $K_{mf}$ have been lumped together into the single term, $K_{mt}$, in this model.

### 4.2.3 Testing the Model

To establish the validity of the model over the dose range of the simulation, it was first tested against the multiple dose steady state data of Walle *et al.*(14). Simulated single dose data agreed with their observations in that tripling the simulated single dose peak accurately predicted steady state peak concentrations (Figure 4-2). In addition, for each dosage level used in the study (0.25, 0.5 and 1.0 mg/kg), twelve pulses (corresponding to oral drug administration) were generated at 6 h intervals to produce a steady state. The peak and trough steady state concentrations fell within the bounds established by Walle *et al.* (14). From these data it was concluded that the pooled Michaelis-Menten parameters calculated by Wagner (13) were suitable for use in the model.

### 4.2.4 The Effects of Dose

Because saturation kinetics may play a role in the food effect, the patterns of parameter changes described below were repeated at three doses, 0.25 , 0.5 and 1 mg/kg, roughly corresponding to 20, 40 and 80 mg single doses of propranolol, respectively, administered to an average adult.

### 4.2.5 The Effects of Changes in $Q_h$, $K_{mt}$, or $V_{max}$ and Their Duration

The relative effects of equivalent changes in $Q_h$, $K_{mt}$ and $V_{max}$ on AUC were simulated to test the sensitivity of the system to changes in each parameter. Each parameter was changed by a factor of two from its base value to give an increase in AUC. This relative magnitude of change was used because the maximum measured post-prandial increase in $Q_h$ was about 100% for humans (7) and this value was close to the minimum increase observed in the dog (20). For comparison, the other two

parameters were changed by the same relative degree. To produce an increase in AUC, $K_{mt}$ was doubled or $V_{max}$ was halved.

The effects of a meal are transient and extremely variable, depending on the composition of the meal as well as the individual. Studies have shown that the duration of increased $Q_h$ was approximately 190 min in humans (7) and 3-7 h in the dog (20) after high protein meals. The alteration in $Q_h$ was used as a basis for comparing the effects of duration of changes to all three parameters on AUC. Not only were the three parameters separately changed for the duration of a simulation, but they were changed transiently in a pattern meant to mimic the pattern of a food effect. Each parameter was separately changed from its base value according to a form which followed the estimated rise and fall in hepatic blood flow (7, 20, Fig. 4-3). The duration of this pattern was set at 90, 180 or 360 min to encompass the the observed range in biological variability in humans and dogs. While this sort of pattern may not be completely valid for $K_{mt}$ and $V_{max}$, in the absence of data to the contrary, it serves as a basis for comparison between parameters.

### 4.2.6 The Effect of Varying Input Rate

Propranolol is administered in a regular release dosage form and a sustained release dosage form. To mimic the release patterns of both, simulations were performed with $K_a$ set at 0.01 and 0.002 $min^{-1}$, which gave peak concentration times in the systemic compartment of 2.5 and 5 h, respectively, under the standard condition.

## 4.3 Results and Discussion

### 4.3.1 The Effect of Dose

It has been mentioned (13) that during the absorption process, high liver propranolol concentration can saturate metabolism temporarily, but the magnitude of this effect has not been evaluated. The model indicates that liver concentrations may remain in excess of $K_{mt}$ for 2.5 h when a standard 1 mg/kg dose is given (Fig 4-4). Therefore the hepatic enzymes are at least partially saturated during most of the absorptive phase. At a dose of 0.5 mg/kg, the period when liver concentration is above $K_{mt}$ is much shorter, and the concentration reached is much lower, the extraction ratio drops transiently to about 0.65. At a dose of 0.25 mg/kg, the liver concentration remains below $K_{mt}$ and the kinetics are nearly linear. It is shown in Table 4-1 that the the AUC after 0.5 mg/kg is only slightly more than double that after 0.25 mg/kg, but after 1.0 mg/kg, the AUC is more than triple that after 0.5 mg/kg.

The standard conditions with Michaelis-Menten equations installed give some interesting insights into how saturation of metabolism may play a large role in determining the bioavailability of a drug subject to the food effect, even though the peripheral venous drug concentration is far below $K_{mt}$. At the beginning of drug absorption, portal venous and liver concentrations are very high (Fig. 4-4). The extraction ratio and clearance drop accordingly. E reaches below 0.5 and Cl falls below 10 ml/min/kg (Fig. 4-5). As the dose is absorbed and liver concentrations fall, E gradually climbs to about 0.85 as absorption approaches completion. $Cl_h$ reaches 17 ml/min/kg. The importance of this observation can be realized when one considers that under dynamic conditions, hepatic clearance is constantly changing over a wide interval. The AUC method of bioavailability comparison used so frequently in the literature assumes clearance to be constant. For high first pass drugs exhibiting non-

linear kinetics, this method of comparing dosage form bioavailability may be invalid because AUC is not necessarily directly related to the amount of drug available to the systemic compartment. Several groups have introduced and evaluated methods which can more accurately estimate the bioavailability of drugs showing capacity-limited elimination (21 - 24). Further development of this area is required.

### 4.3.2 The Effect of Changing Parameters

#### 4.3.2.1 $Q_h$

It can be seen in Table 4-1 that the model predicts a slight (<10%) increase in AUC when a 1.5 or 3.0 h pattern of increased $Q_h$ is used at a dose level of 1 mg/kg. With a duration of change of longer periods, a decrease in AUC is predicted. During the absorptive phase, $Q_h$ does not bring about as large an increase in bioavailability as is seen with linear models because partial saturation of the enzymes decreases the sensitivity of the system to $Q_h$ changes. During much of the elimination phase, when the system is less saturated, the increase in $Q_h$ causes an increase in Cl. Under less saturated conditions at lower dose levels, proportionately larger AUC changes are predicted, which agree better with the linear models. It is interesting to note that under linear conditions, where intrinsic clearance is expressed as $V_{max}/K_m$, the model predicts a 22% increase in AUC between the standard condition and the 1.5 h $Q_h$ increase pattern. This contrasts with the Michaelis-Menten model which predicts only a 7% increase. This lack of sensitivity to $Q_h$ changes during the absorptive phase may help to explain why Modi *et al.* (9) observed no effect of $Q_h$ on AUC after an 80 mg dose of propranolol.

#### 4.3.2.2 $K_{mt}$

The effects on the model of doubling $K_{mt}$ are seen in Table 4-1. At low doses, the system is generally more sensitive to changes in these parameters, because capacity limitation is playing a smaller role. Only at a dose of 1 mg/kg with parameter variation over 1.5 h was the predicted increase in AUC less than 10%. As the duration of parameter change increased, the AUC increased. The model shows intermediate sensitivity overall to changes in $K_{mt}$.

$K_{mt}$ can be affected by changes to both $K_{mf}$ and $f_b$. An increase in $K_{mf}$ may occur as a result of competition between drug and nutrients for enzyme or binding sites. Conversely, displacement of drug from plasma protein binding sites would increase availability of drug to the metabolic enzymes, reducing the $K_{mt}$. These mechanisms have not been well studied and their roles in the food effect have not been delineated. Competition for metabolic enzyme binding sites would be expected to cause an increase in $K_{mf}$. In our laboratory, parenteral nutrients administered to pigs reduced lidocaine clearance by approximately 50%, indicating an increase in $K_{mf}$ (25).

Binding of drug to plasma proteins such as albumin and $\alpha_1$-acid glycoprotein could also be affected by nutrients. To bring about an increase in AUC, however, binding would have to increase in the presence of nutrients. Woo and Greenblatt (26) have shown a slight postprandial increase in quinidine binding from 72.5% to 76.8% , which is not enough to cause a significant change in bioavailability. There is little evidence for this effect on binding of other drugs. More likely would be the displacement of lipophilic drugs from binding sites by ingested lipid, increasing $f_b$ which in turn would lower AUC. For instance, in our laboratory, it was found that lidocaine binding decreased by 15% when parenteral nutrients were given to swine (J. Ke, Y. K. Tam, H. A. Semple and R. T. Coutts, unpublished data). Nutrient effects

on $f_b$ and tissue binding could be opposite to those caused by other mechanisms, complicating the food effect mechanism.

#### 4.3.2.3 $V_{max}$

The dramatic increases in AUC caused by halving $V_{max}$ are illustrated in Table 4-1. Even with only a 1.5 h change pattern, the least sensitive dose, 1 mg/kg shows a 30% increase in AUC. These simulations show that the model is twice to three times as sensitive to changes in $V_{max}$ compared to equivalent changes in $K_{mt}$ (Fig. 4-6, 4-7). The model is least sensitive to $Q_h$ changes. Increasing the duration of change of $K_{mt}$ and $V_{max}$ increases the magnitude of the change in AUC. Since the dog shows in general a longer duration of the food effect than does man, it may be a good animal model. The effects of probes used to isolate each mechanism will be more easily seen if they are enhanced by a greater duration.

Any mechanism which changes the quantity of metabolic enzymes available to the drug will change $V_{max}$. Several unrelated mechanisms may have this effect, either directly or indirectly. Direct mechanisms may act on the enzymes themselves. The role of hormonal regulation of short-term enzyme activity has long been recognized for the enzymes of the glycolytic pathway, but recently, a role for insulin in the deactivation of enzymes involved in aromatic amino acid metabolism (27), and activation of cytochrome p-450 metabolism of androstenedione (28) has been demonstrated. The effects on metabolism were observed within 0.5 h in both studies. We speculate that since insulin and glucagon are intimately involved in the food effect, they and other hormones such as the glucocorticoids and catecholamines may be involved in regulating metabolic enzyme activities in the presence of oral nutrients.

Other direct mechanisms are possible. Concentrations of cofactors or other molecules necessary for metabolic enzyme activity could be altered by food, changing

enzyme activity and hence $V_{max}$. Yang and Yoo (29) pointed out that the supply of NADPH, which is the electron donor for cytochrome p-450, is dependent on the availability of glucose-6-phosphate, which in turn is related to the blood glucose level, glycogen storage and gluconeogenesis. In addition, in situations with elevated cytochrome p-450 activity and high doses of substrates (these could be present in food), the NADPH supply can limit mixed function oxidase activity. Levy has presented a general discussion on the role of endogenous co-substrate supply in mediating metabolic activity (30).

Indirect mechanisms involving restriction of access to enzymes by drug may also exist. Even lipophilic drugs could be uptake rate limited, especially if nutrient competition exists at uptake sites. Also, shunting of a portion of portal blood past the liver has been mentioned as a possible mechanism (10). It should be pointed out that all the mechanisms discussed in this section could produce global changes in apparent mixed function oxidase activity, so that the lack of an effect on a specific propranolol pathway seen by Walle *et al.* (31) is not surprising.

### 4.3.3 The Effect of Changing Input Rate

The effect of different input rates on first-pass drug bioavailability has been the subject of several studies (13, 32, 33, 34) . Both regular release and sustained release profiles have been modelled using Michaelis-Menten equations and found to agree with human data which show that bioavailability of sustained release dosage forms is less than that of regular release formulations. Figure 4-7 shows that the model agrees with the earlier studies. The model was less sensitive to changes in any of the parameters, especially $V_{max}$ when a $K_a$ representative of sustained release was used (Fig. 4-7, 4-8). It is unlikely that changes in $Q_h$ would cause significant changes in AUC, while changes in $K_{mt}$ or $V_{max}$ would have to be prolonged to be observable. The main reason for the

model's decreased sensitivity at low input rates is that the parameter changes cover only a portion of the absorption phase.

## 4.4 Conclusions

1. High liver concentrations of drug during oral absorption can cause saturation of enzymes at moderate propranolol doses, resulting in reduced extraction during the absorptive phase.
2. The system is moderately sensitive to $K_{mt}$, but most sensitive to $V_{max}$ changes. Considering the magnitude of the food effect, important mechanisms involving large changes to $V_{max}$ likely remain to be discovered.
3. Reduced sensitivity to hepatic blood flow changes occurs with Michaelis-Menten kinetics, compared with linear kinetics, at any duration of parameter change. The system becomes more sensitive to changes in $K_{mt}$ and $V_{max}$ as the duration of the change increases.
4. In agreement with earlier studies, a slower input rate decreases AUC for a given dose. The model also supports the suggestion that at slow input rates representative of sustained release, the effect of food on AUC is also decreased.

## 4.5 Table

Table 4-1. Physiological model with capacity-limited first pass metabolism: simulations of possible food effects on propranolol AUC through changes to $Q_h$, $K_{m_t}$ or $V_{max}$.

| CONDITION | AUC (ng.min/ml) | | | % CHANGE | | |
|---|---|---|---|---|---|---|
| DOSE (mg/kg) | 1.00 | 0.50 | 0.25 | 1.00 | 0.50 | 0.25 |
| $Q_h$=20 ml/min/kg | 26371 | 8010 | 3095 | 0 | 0 | 0 |
| $Q_h$=40 ml/min/kg | 22745 | 7477 | 3027 | -13.75 | -6.65 | -2.20 |
| $Q_h$=Varied over 1.5 h | 28228 | 9284 | 3718 | 7.04 | 15.91 | 20.13 |
| $Q_h$=Varied over 3 h | 28682 | 9363 | 3753 | 8.76 | 16.89 | 21.26 |
| $Q_h$=Varied over 6 h | 26289 | 8236 | 3251 | -0.31 | 2.82 | 5.04 |
| $K_m$= 50 ng/ml | 26371 | 8010 | 3095 | 0 | 0 | 0 |
| $K_m$= 100 ng/ml | 38573 | 13942 | 5850 | 46.27 | 74.06 | 89.01 |
| $K_m$=Varied over 1.5 h | 28752 | 9691 | 3929 | 9.03 | 20.99 | 26.95 |
| $K_m$=Varied over 3 h | 30847 | 10683 | 4371 | 16.97 | 33.37 | 41.23 |
| $K_m$=Varied over 6 h | 33337 | 11530 | 4723 | 26.42 | 43.95 | 52.60 |
| $V_{max}$=5000 ng/min/kg | 26371 | 8010 | 3095 | 0 | 0 | 0 |
| $V_{max}$=2500 ng/min/kg | 61237 | 19287 | 6971 | 132.21 | 140.79 | 125.23 |
| $V_{max}$=Varied over 1.5 h | 34321 | 11395 | 4269 | 30.15 | 42.26 | 37.93 |
| $V_{max}$=Varied over 3 h | 39683 | 13004 | 4784 | 50.48 | 62.35 | 54.57 |
| $V_{max}$=Varied over 6 h | 44665 | 13819 | 5053 | 69.37 | 72.52 | 63.26 |

Standard conditions set at: $K_a$ = 0.01/min, $Q_h$ = 20 ml/min/kg B. W., $K_{m_t}$ = 50 ng/ml, and $V_{max}$ 5000 ng/min/kg

## 4.6 Figures

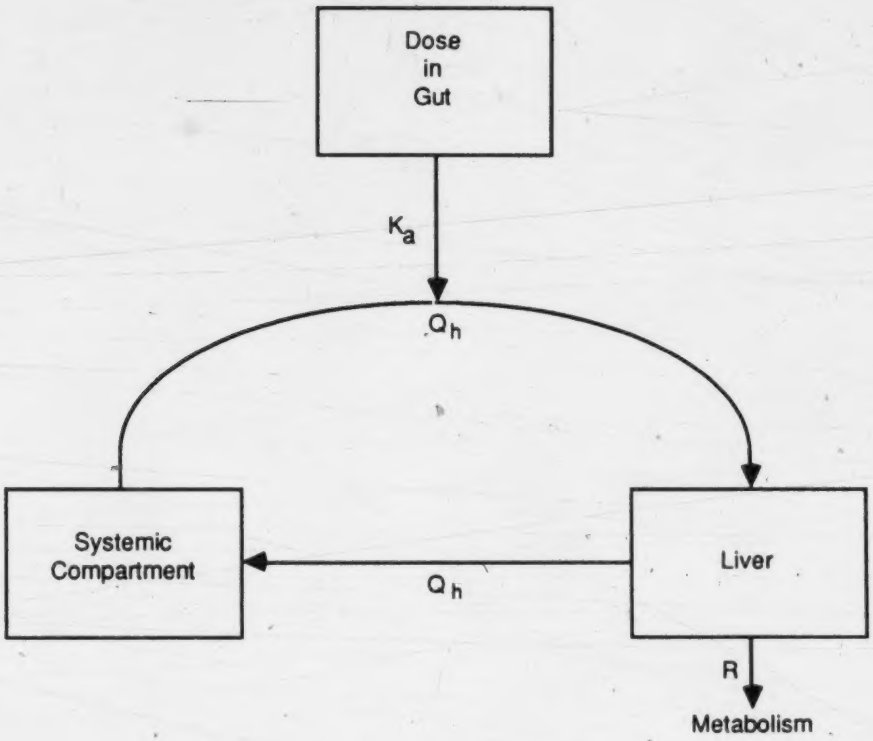


Figure 4-1. Flow diagram of a three compartment physiological model describing propranolol disposition after an oral dose.

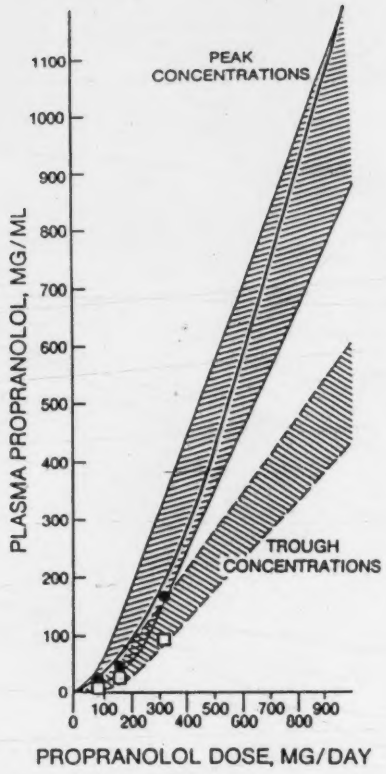


Figure 4-2. General relationship between peak and trough plasma concentrations and dose (from T. Walle, E. C. Conradi, U. K. Walle, T. C. Fagan and T. E. Gaffney, The predictable relationship between plasma levels and dose during chronic propranolol therapy. *Clin. Pharmacol. Ther.* 24:668 (1978), used with permission). Superimposed are a) a line showing the relationship between steady state peak concentration predicted from simulated single dose peak concentrations and dose, and b) simulated steady state peak (filled circles) and trough (open squares) concentrations after 12 doses of 0.25, 0.5 and 1.0 mg/kg given every 6 h. All points fall within the prescribed boundaries.

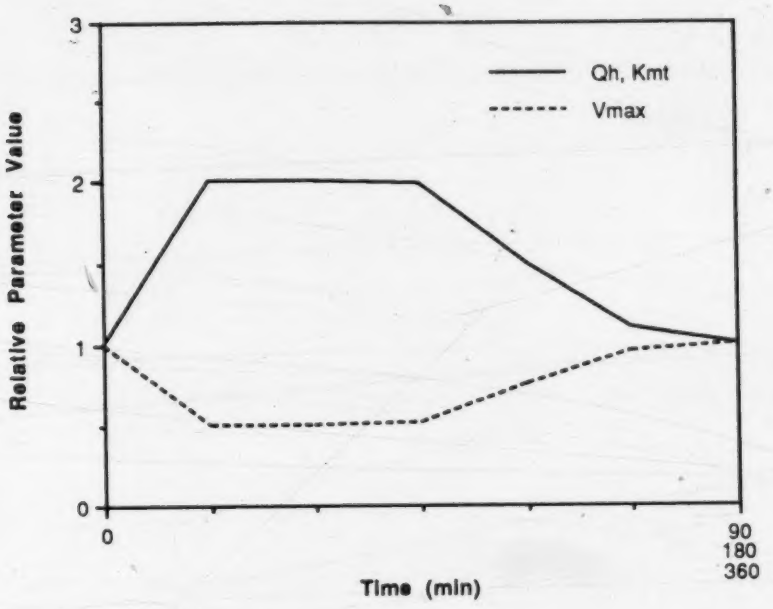


Figure 4-3. Patterns of variation of $Q_h$, $K_{mt}$ and $V_{max}$ from base levels with time for the three durations studied.

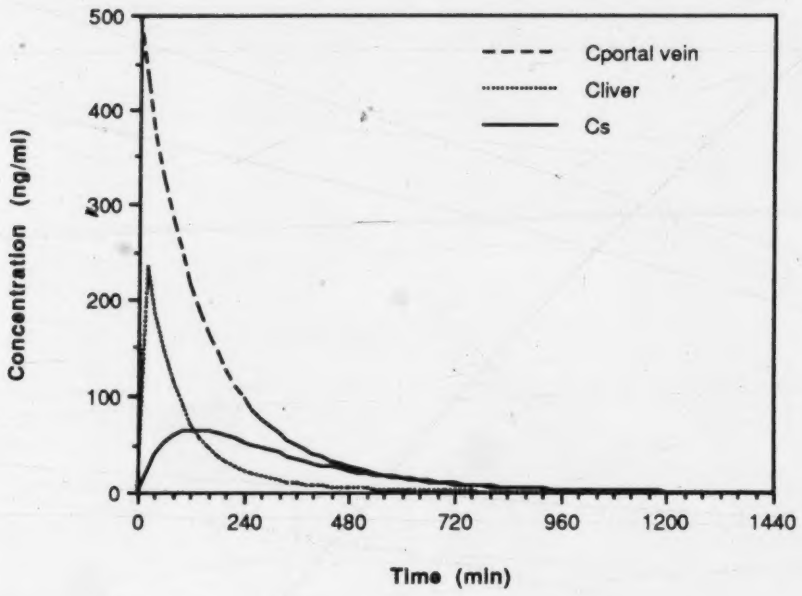


Figure 4-4. Propranolol concentrations in the portal vein, liver, and systemic compartment *vs.* time for the Basic Model after a 1 mg/kg dose. Note that $C_{liver}$ is above $K_{mt}$ (50 ng/ml) for the first 150 min of the simulation, causing saturation during the period of maximum absorption. The systemic compartment concentration, however, barely exceeds $K_{mt}$.

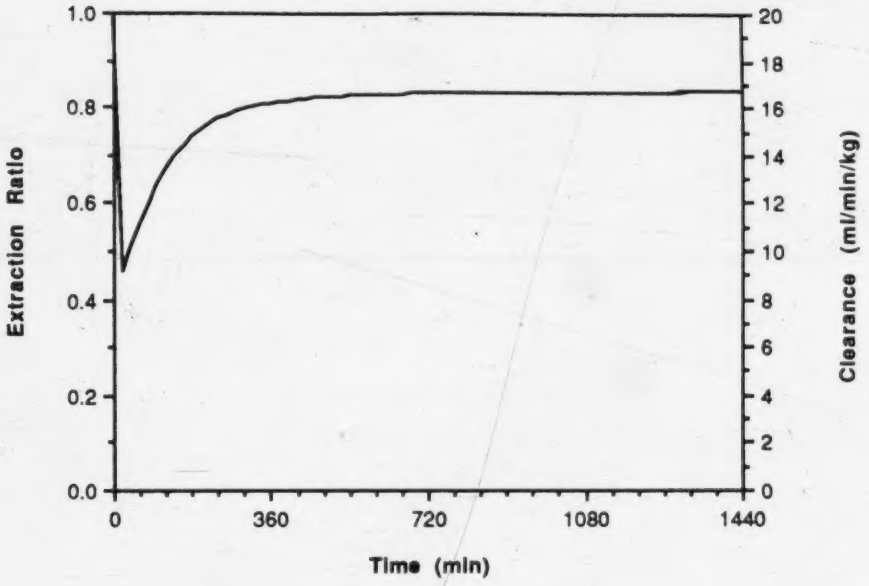


Figure 4-5. Extraction ratio and clearance *vs.* time for the Basic Model after a 1 mg/kg dose. Saturation causes depression of both during most of the absorptive phase.

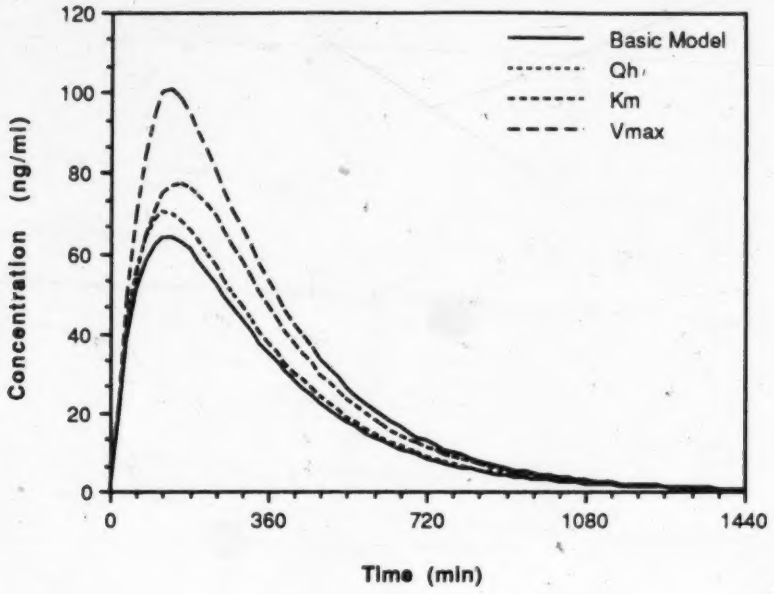


Figure 4-6. Simulated concentration *vs.* time curves showing the results of 180 min transient patterns of variation of $Q_h$, $K_{mt}$ and $V_{max}$ compared with the Basic Model after a 1 mg/kg dose. $Q_h$ variation causes little change, $K_{mt}$ variation causes moderate change, while $V_{max}$ variation causes the highest change from the Basic Model profile.

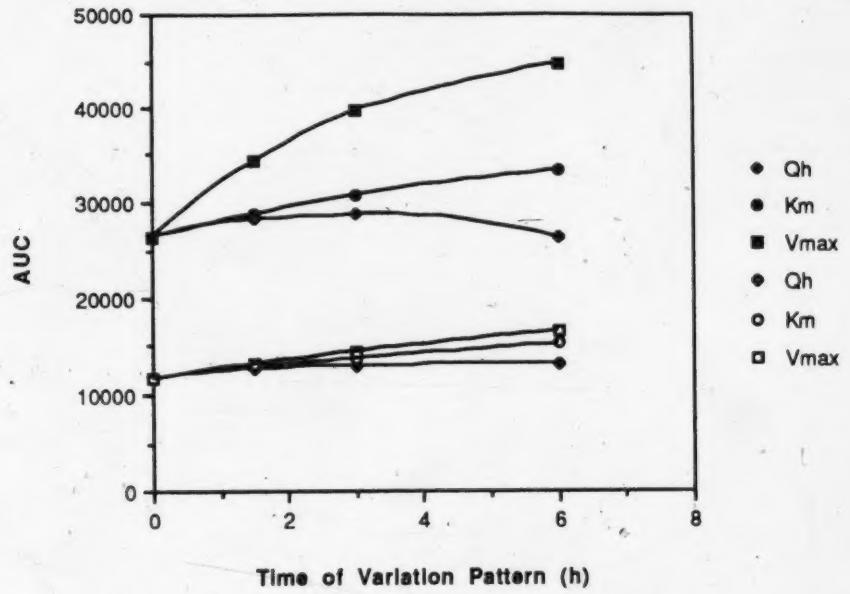


Figure 4-7. Effect of $Q_h$, $K_{mt}$ and $V_{max}$ variation pattern duration on AUC for regular ($K_a$ = 0.01 $min^{-1}$, closed symbols) or sustained release ($K_a$ = 0.002 $min^{-1}$, open symbols) propranolol.

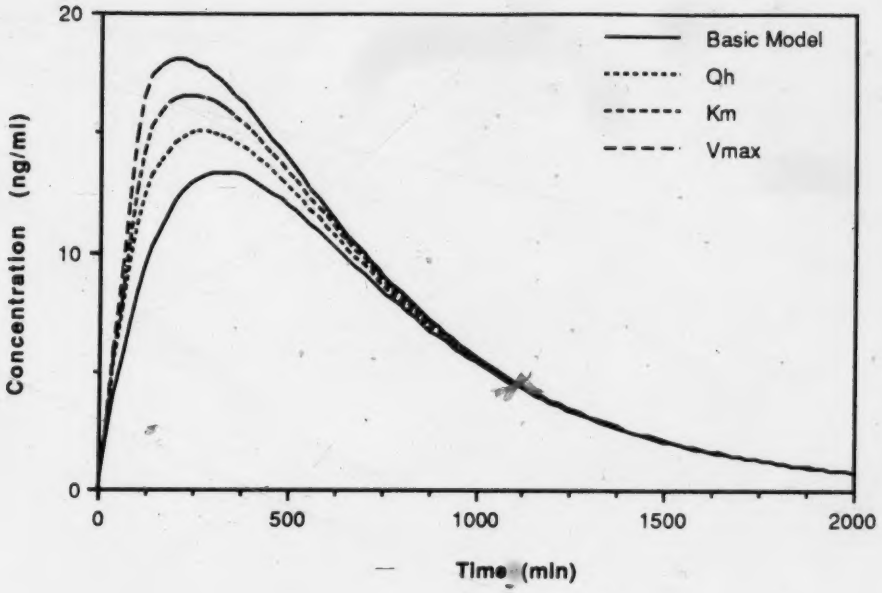


Figure 4-8. Simulated concentration *vs.* time curves for sustained release ($K_a$ = 0.002 $min^{-1}$), showing the results of 180 min transient patterns of variation of $Q_h$, $K_{mt}$ and $V_{max}$ compared with the Basic Model after a 1 mg/kg dose. Note the differences in scale between this and Figure 4-6, and that the changes brought about by parameter variation are smaller than those in Figure 4-6.

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# 5. Hydralazine Pharmacokinetics and Interaction With Food: An Evaluation of the Dog as an Animal Model[1]

## 5.1 Introduction

Hydralazine has been used as a peripheral vasodilator in humans for many years but extreme variability in oral dose requirements has limited its usefulness (1). Its disposition is partly dependent on acetylator phenotype (1-3), but much of the variability in oral dosage requirements may be mediated by other factors influencing the first pass effect, such as splanchnic blood flow, protein binding and hepatic/intestinal metabolic capacity. Contributing to this variability may be an interaction with food. Melander *et al.* (4) and Liedholm *et al.* (5) reported data which suggested that the bioavailability of hydralazine was increased when co-administered with food. Subsequently, however, Shepherd *et al.*, using a more specific assay, reported a 46% decrease in hydralazine AUC when taken 45 min after a meal (5) and in another study, a 33% decrease when hydralazine was consumed immediately after a meal (7). They pointed out that the assays employed by Melander *et al.* (4) and Liedholm *et al.* (5) were susceptible to interference by hydralazine pyruvic acid hydrazone, an important metabolite which may hydrolyze to produce free hydralazine under the acidic conditions employed for derivatization. Studies in humans to elucidate the mechanisms of the food-hydralazine interaction would be difficult to perform so an animal model would be useful.

The dog has been developed successfully as a model for first pass propranolol elimination using invasive techniques, and the effect of hydralazine on propranolol

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[1]A version of this chapter has been accepted for publication. H. A. Semple, Y. K. Tam and R. T. Coutts. *Pharm. Res.* (1989).

disposition has been studied (8, 9). The dog possesses additional characteristics which make it an attractive species in which to study hydralazine pharmacokinetics. An early study using a non-specific assay found the pattern of absorption and elimination in dogs to be similar to that in humans (10). In the same study, the binding of radiolabelled hydralazine to plasma was found to be slightly lower in dogs than in humans (71% *vs.* 87%), and the partitioning into red cells was slightly higher in dogs than in humans (0.37 *vs.* 0.25). The pharmacodynamic effect on dogs is similar to that in humans, with peripheral vasodilation causing reduced cardiac afterload (11), and hydralazine has been used to treat chronic left heart failure in dogs (11 - 13). Hepatic blood flow after hydralazine administration is increased in the dog in a qualitatively similar pattern to that in humans (6, 8, 14).

While the dog is reputed to be a poor acetylator of aromatic amines, poor acetylation ability could be an advantage in studying some of the other mechanisms of hydralazine elimination, because a major cause of variability would be missing, allowing the investigator to focus on the others (15). The value of this approach has been recognized by Ludden *et al.* (16) who recently published a hydralazine bioavailability study in which only slow acetylating humans were evaluated in order to decrease inter-subject variability in kinetic parameters. Little is known about the kinetics of hydralazine in the dog, however, and more specific information is required to evaluate the dog's usefulness as an animal model.

The study described here was designed to provide basic intravenous and oral pharmacokinetic data on hydralazine in the dog for comparison with human data. Different doses were used to determine if dose-dependent elimination occurs after oral hydralazine administration, as has been observed in humans (17). In addition, a meal study was performed to determine if a pharmacokinetic interaction occurs between hydralazine and food in dogs.

## 5.2 Materials and Methods

### 5.2.1 Reagents and Chemicals

Acetonitrile and hexane were HPLC-grade solvents (Caledon, Edmonton, Alberta). (1,4)-Dioxane was glass distilled and p-nitrobenzaldehyde was recrystallized from ethanol before use. Hydralazine hydrochloride (Sigma Chemical Co., St. Louis, MO), 4-methylhydralazine, (Ciba-Geigy Co., Basel, Switzerland), triethylamine and phosphoric acid, 85% (Fisher Scientific Co., Fairlawn, N.J.) were used as received.

### 5.2.2 Pharmacokinetic Studies in Animals

Four random source, cross bred dogs weighing between 12 and 40 kg were used. After an overnight fast, a Teflon catheter was inserted into a cephalic vein for blood sampling, a rubber septum was attached, a blank blood sample was collected into a syringe containing EDTA for standard curve preparation and a heparin lock was used to maintain catheter patency. Intravenous doses of hydralazine HCl (Apresoline) were administered into the opposite cephalic vein. Oral doses consisted of the appropriate volume of Apresoline solution diluted with 0.01 N HCl to fill a 60 ml syringe. The dilute solution was carefully administered to the dog orally over about 1.5-2 min. Doses were given in random order and route at intervals of not less than one week, to allow drug washout. Four doses were used: 0.25, 1.0, 2.5 and 4.0 mg/kg. After intravenous dosing, 1 ml blood samples were collected at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480 min, and for 2.5 and 4.0 mg/kg doses, additionally at 540, 600, 660 and 720 min. After oral dosing, samples were collected at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 135, 150, and so on as for the low intravenous doses. For the meal study, the dogs were fasted overnight, given a 2.5 mg/kg oral dose of hydralazine HCl, then immediately given

their normal daily maintenance ration of a commercial canned dog food (~25 g/kg of Science Diet Maintenance, Hills). This was consumed in less than two min in all cases. Sampling then followed the normal oral schedule.

### 5.2.3 Assay Method

Hydralazine was assayed in blood using a slight modification of a previously described HPLC method (18). The blood sample size used was 1 ml and the volume of hexane used for extraction was 5 ml instead of 3 ml. Each extraction residue was dissolved in 25 µl of acetonitrile instead of 150 µl of mobile phase. The retention time of the hydralazine derivative was 3.4 min and of the internal standard derivative, 4.6 min. Standard curves were prepared fresh from the blank blood samples collected from each dog at the beginning of each experiment. The standard curves were linear over the concentration range 2 to 5000 ng/ml. Quintuplicate quality control samples were independently prepared to test the accuracy and precision of the method at 2 and 5000 ng/ml. They were analyzed to contain $1.9 \pm 0.3$ ng/ml and $4620 \pm 203$ ng/ml, respectively.

### 5.2.4 Pharmacokinetic Analysis

The concentration *vs.* time data were fit using LAGRAN (19). The pharmacokinetic parameters, $Cl_{tb}$, $Cl_{oral}$, AUC, $V_{ss}$ and F were calculated according to published methods (20). $C_{max}$ and $t_{max}$ were taken directly from the concentration *vs.* time data.

### 5.2.5 Statistical Analysis

A crossover design was used. Comparison of dose groups was accomplished using a repeated-measures analysis of variance, with $\alpha = 0.05$. The Scheffé test ($\alpha = 0.05$) was used to compare means. The significance of trends in the data was established by

testing the slopes using simple linear regression ($\alpha = 0.05$). The $AUC_{oral}$ of hydralazine under fasted and fed conditions was compared using the Wilcoxon Signed Rank Test ($\alpha = 0.05$).

## 5.3 Results

### 5.3.1 Intravenous Data

Log hydralazine concentration *vs.* time curves are shown in Figures 5-1 to 5-4 for the four intravenous doses in each dog, and the resulting pharmacokinetic parameters are summarized in Table 5-1. There were no significant differences among dose groups for total body clearance, AUC/dose, $V_{ss}$ or $\beta$. Mean $Cl_{tb}$ was 70 ml/min/kg, the mean $V_{ss}$ was 9 *l*/kg, and the mean $\beta$ was 0.0044 $min^{-1}$.

### 5.3.2 Oral Data

Figures 5-5 to 5-8 show the log concentration *vs.* time curves for the four oral doses of Apresoline liquid in the 4 dogs. There are secondary peaks in most of the individual oral curves, occurring from about 75 min to 420 min after dosing. Most curves contained at least one secondary peak, and some contained two or three.

Significant differences in oral AUC/dose and F were observed between the 0.25 and 4 mg/kg doses. Linear regression indicated that there were significant trends toward higher AUC/dose and F with increasing dose (Figures 5-9, 5-10). $Cl_{oral}$, $\beta$ (Table 5-1) and $C_{max}$/dose were not significantly dose related. The mean slopes of the terminal elimination phases were not significantly different from those after iv doses (Table 5-1). The time to peak concentration ($t_{max}$) was quite variable (range 5-45 min, Table 5-1).

### 5.3.3 Meal Effect Data

Mean (± SE) concentration *vs.* time curves are presented in Figure 5-11. Fasted and postprandial pharmacokinetic parameters of hydralazine are compared in Table 5-2. A significant (mean 63%) reduction in hydralazine AUC was observed when an oral 2.5 mg/kg dose was accompanied by a meal (Table 5-2). Apparent oral clearance was tripled (Table 5-2). The mean $C_{max}$ was apparently halved, but this decrease was not statistically significant due to the high variability in this parameter. The $t_{max}$ and $\beta$ values were not significantly different between the two conditions.

## 5.4 Discussion

Previously reported human pharmacokinetic data are summarized in Table 1-1 for comparison with the canine data from this study in Tables 5-1 and 5-2. The total body clearance of hydralazine in the dog (Table 5-1) is similar to that in humans (Table 1-1), and does not change over the intravenous dose range examined. This parameter is about 3-4 times resting hepatic blood flow, indicating the existence of significant extrahepatic pathways. The rate of hydralazine pyruvic acid hydrazone production in canine blood has not yet been reported, but the high clearance value is indirect evidence that this metabolic pathway could be as important in the dog as it is in the human.

$V_{ss}$ of hydralazine in the dog (Table 5-1) may be somewhat larger than in the human (Table 1-1), although the ranges of individual estimates overlap (3.0-14.1 *l*/kg, dog *vs.* 1.3-7.9 *l*/kg, human (3, 21)). This could, in large part, account for the smaller $\beta$ values observed in the dog.

The occurrence of the secondary peaks in the oral concentration *vs.* time profiles may indicate that hydralazine undergoes some enterohepatic recirculation. This phenomenon would be much less important after an intravenous dose because with the large volume of distribution of hydralazine in the dog, the concentration of drug

reaching the liver and hence the rate of biliary excretion would be much lower. The intravenous curves would therefore not be visibly perturbed by this process. Another explanation for the secondary peaks could be that the rate of hydralazine absorption varies along the length of the gastrointestinal tract. While this phenomenon has not been mentioned in the literature on humans, some smaller fluctuations in concentration can be seen in the individual oral profiles reported by Shepherd *et al.* (22). The presence of these peaks warrants further investigation.

The trend towards rising oral dose-normalized AUC with increasing dose may indicate saturation of first pass metabolism (Figure 5-9). This is consistent with observations in hypertensive humans (17). The dose-dependency of hydralazine F in the dog (Figure 5-10) supported the AUC/dose data in suggesting saturation of first pass metabolism at therapeutic doses. Although Michaelis-Menten kinetics may operate at the doses used, the conventional AUC method of determining F may be reasonably accurate for hydralazine because this drug is rapidly absorbed and has a large $V_{ss}$ (23-25). $C_{max}$ values are more variable in dogs (Table 5-1), than those in humans (Table 1-1), although the ranges overlapped between dogs and slow acetylating humans at both the 0.25 and 1.0 mg/kg dose levels. The high variability in $C_{max}$ could partially explain why $C_{max}$/dose *vs.* dose were not significantly related. However, a similar trend to that in humans was observed. Values for $t_{max}$ after administration of the liquid formulation were similar in the dog and human (Tables 5-1, 1-1), averaging around 20 min in both species.

The meal study (Table 5-2, Figure 5-11), revealed that, in agreement with the human data of Shepherd *et al.* (6, 7), co-administration of hydralazine with food caused a reduction in bioavailability, as measured by AUC changes. These findings are not consistent with the studies by Melander *et al.* (4) and Liedholm *et al.* (5), in which increased bioavailability of hydralazine was observed with concomitant food

administration. $Cl_{oral}$ was increased significantly in the dog, and $C_{max}$ was reduced in 3 of the 4 subjects. Remaining unchanged were $t_{max}$ and $\beta$. It is interesting to note that none of the individual concentration *vs.* time curves showed secondary peaks after a meal. If their origin in the fasted animals was enterohepatic recycling, no peaks would be expected to appear after a meal, because the gall bladder contracts frequently and the small and more or less continuous release of drug into the duodenum is not sufficient to cause a noticeable rise in drug concentration.

It is not possible to use the peripheral blood data to differentiate among the possible mechanisms which may contribute to the food effect on hydralazine bioavailability. These include changes to hydralazine absorption, first pass metabolism, hepatic blood flow and protein binding. Further investigations using invasive methods are being conducted to gain a greater understanding of this interesting, controversial and clinically significant phenomenon.

The data presented here indicate that the parent drug kinetics of hydralazine are quantitatively similar between dogs and slow-acetylating humans. Both species show high systemic clearance indicating significant extrahepatic metabolism, a large volume of distribution, saturation of presystemic metabolism at therapeutic doses and reduced bioavailability of hydralazine when co-administered with food. The dog may therefore be a useful animal model for studying the pharmacokinetics and mechanisms of pharmacokinetic interaction of hydralazine with food.

## 5.5 Tables

Table 5-1. Hydralazine dose dependent data, expressed as means (± SD) from four dogs.

| Dose (mg/kg) | $Cl_{tb}$ (ml/min/kg) | $Cl_{oral}$ (ml/min/kg) | AUC (ng-min/mL) | $V_{ss}$ (l/kg) | $C_{max}$ (ng/ml) | $t_{max}$ (min) | $\beta$ ($min^{-1}$) | $t_{1/2}$ (min) |
|---|---|---|---|---|---|---|---|---|
| Intravenous Doses | | | | | | | | |
| 0.25 | 69 (10) | | 3683 (566) | 10 (5) | | | .0079 (.0075) | 140 (81) |
| 1 | 78 (11) | | 12771 (1560) | 8 (1) | | | .0039 (.0009) | 185 (43) |
| 2.5 | 68 (8) | | 36981 (4192) | 9 (3) | | | .0033 (.0014) | 246 (117) |
| 4 | 67 (20) | | 63697 (18099) | 9 (1) | | | .0025 (.0007) | 290 (99) |
| Oral Doses | | | | | | | | |
| 0.25 | | 314 (312) | 1388 (901) | | 27 (7) | 16 (2) | .0105 (.0119) | 124 (74) |
| 1 | | 164 (76) | 7169 (3179) | | 125 (27) | 11 (1) | .0046 (.0010) | 156 (34) |
| 2.5 | | 135 (37) | 19923 (6727) | | 263 (94) | 27 (7) | .0040 (.0015) | 207 (114) |
| 4 | | 102(69) | 52872 (26632) | | 1186 (424) | 18 (3) | .0033 (.0017) | 262 (147) |

Table 5-2. Mean (± SD) pharmacokinetic parameters from four dogs given 2.5 mg/kg of hydralazine HCl orally without (fasted) or with (fed) food.

| Condition | $Cl_{oral}$ (ml/min/kg) | AUC (ng-min/ml) | $C_{max}$ (ng/ml) | $t_{max}$ (min) | $\beta$ ($min^{-1}$) | $t_{1/2}$ (min) |
|---|---|---|---|---|---|---|
| Fasted | 135 (37) | 19923 (6727) | 263 (187) | 27 (13) | 0.0040 (0.0015) | 207 (114) |
| Fed | 436 (241) | 7348 (3985) | 126 (136) | 26 (17) | 0.0069 (0.0035) | 114 (40) |

## 5.6 Figures

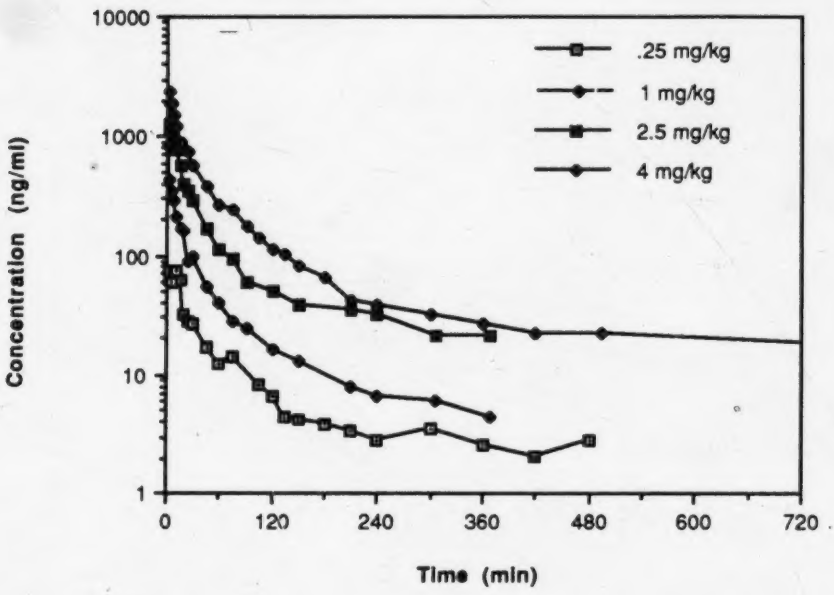


Figure 5-1. Hydralazine concentration *vs*. time profiles of dog 3 after intravenous doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

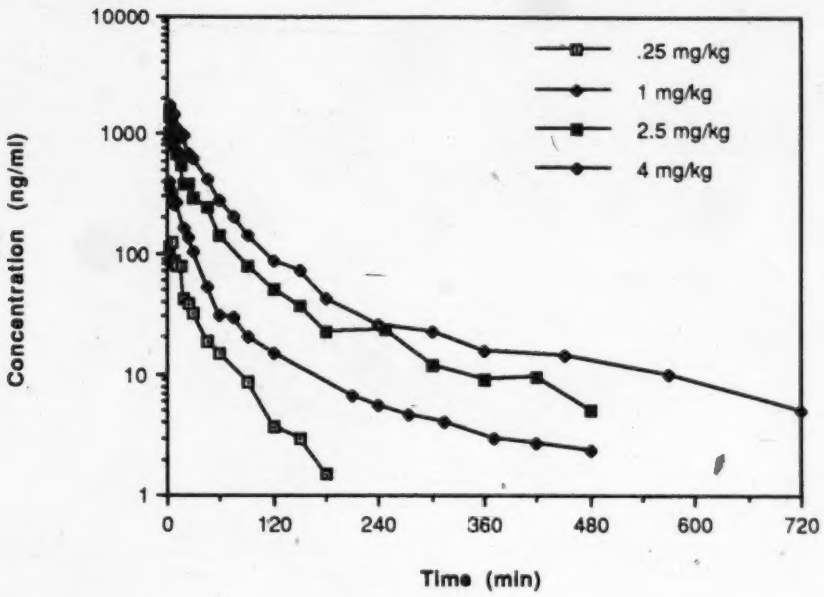


Figure 5-2. Hydralazine concentration *vs*. time profiles of dog 4 after intravenous doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

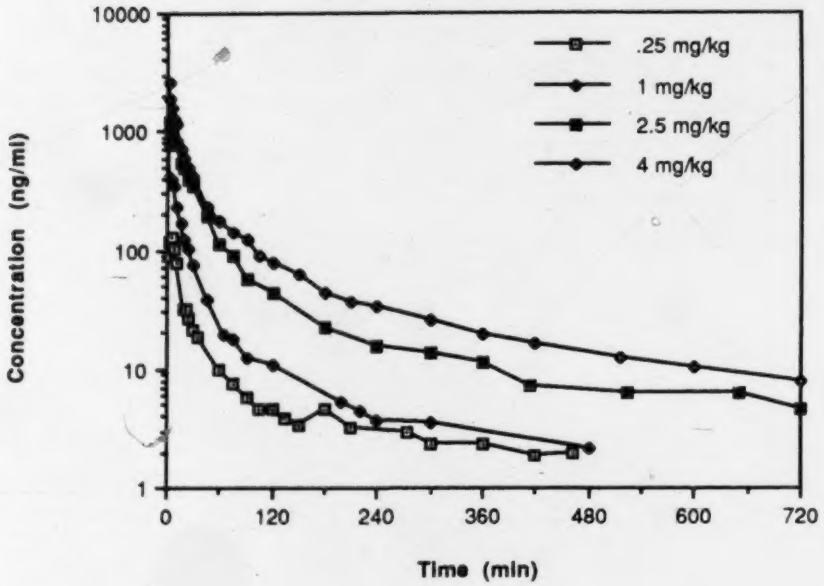


Figure 5-3. Hydralazine concentration *vs.* time profiles of dog 5 after intravenous doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

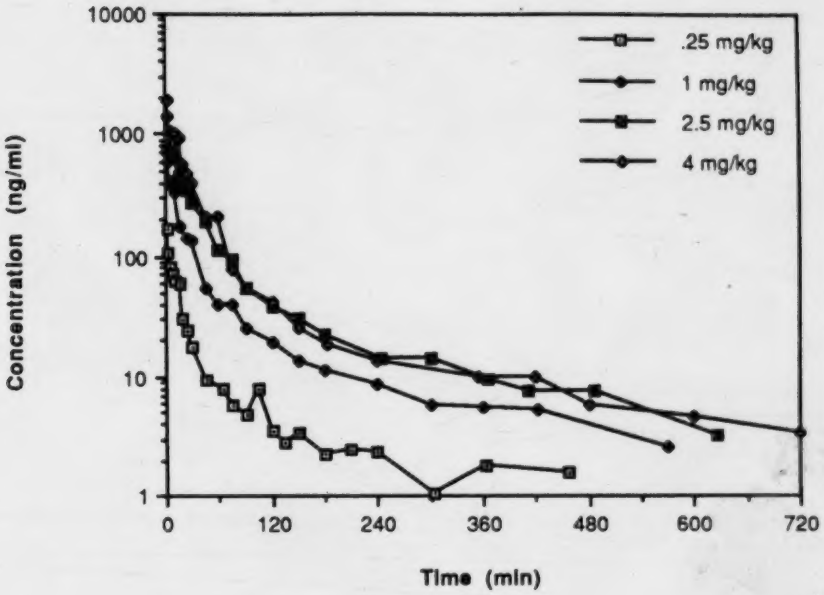


Figure 5-4. Hydralazine concentration *vs.* time profiles of dog 6 after intravenous doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

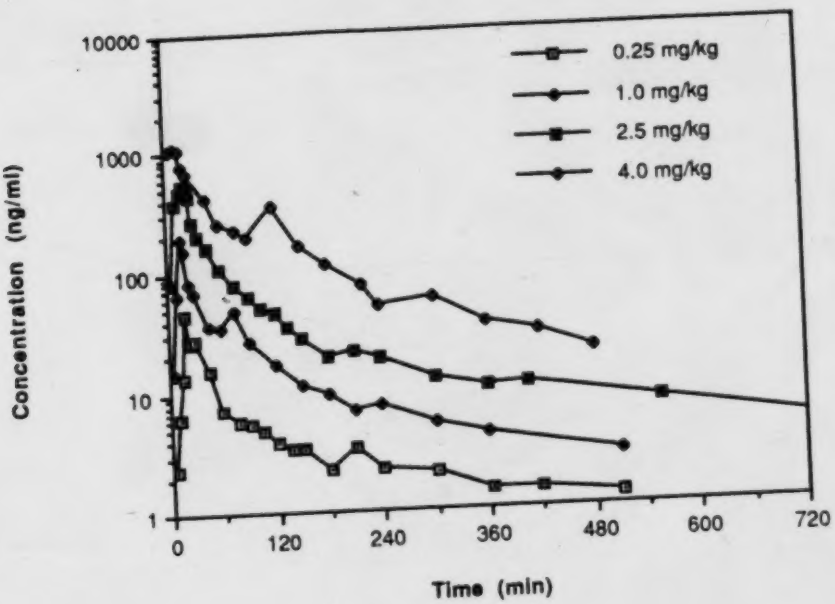


Figure 5-5. Hydralazine concentration *vs*. time profiles of dog 3 after oral doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

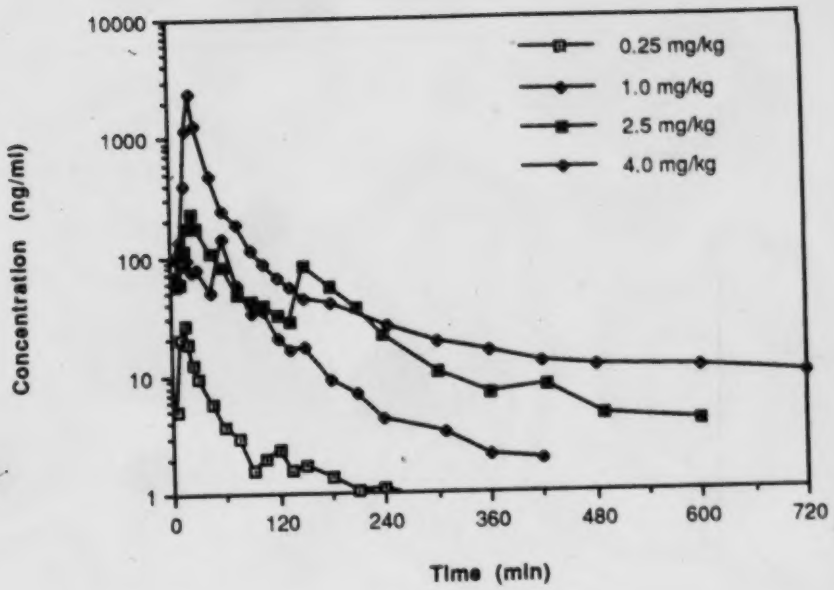


Figure 5-6. Hydralazine concentration *vs.* time profiles of dog 4 after oral doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

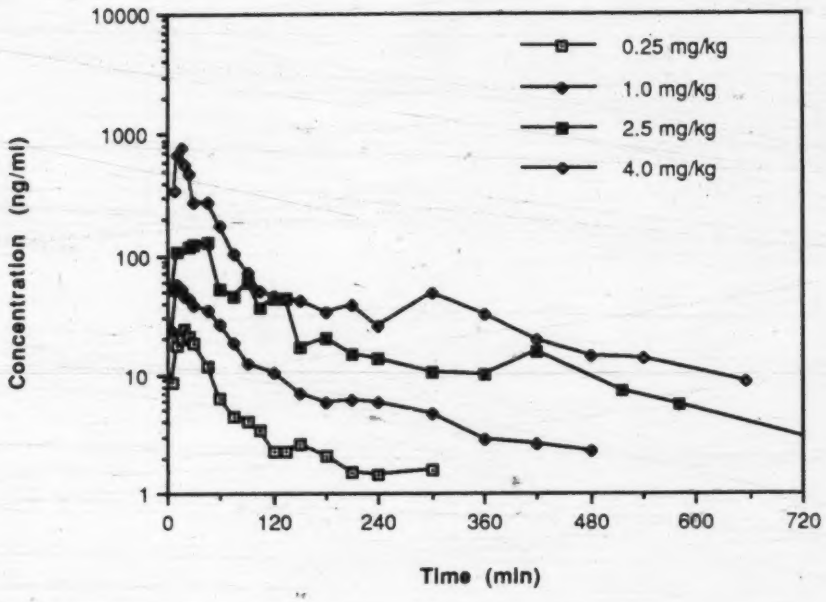


Figure 5-7. Hydralazine concentration *vs.* time profiles of dog 5 after oral doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

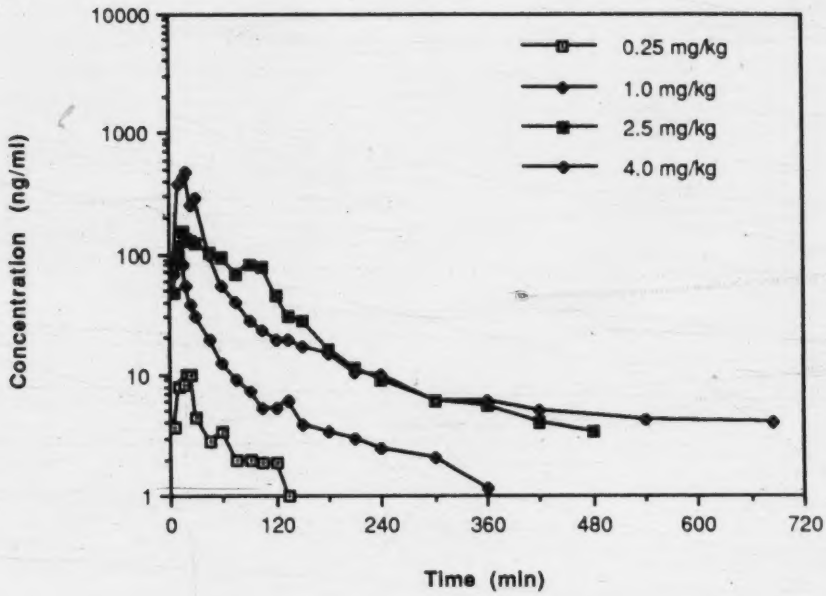


Figure 5-8. Hydralazine concentration *vs.* time profiles of dog 6 after oral doses of 0.25, 1.0, 2.5, and 4.0 mg/kg.

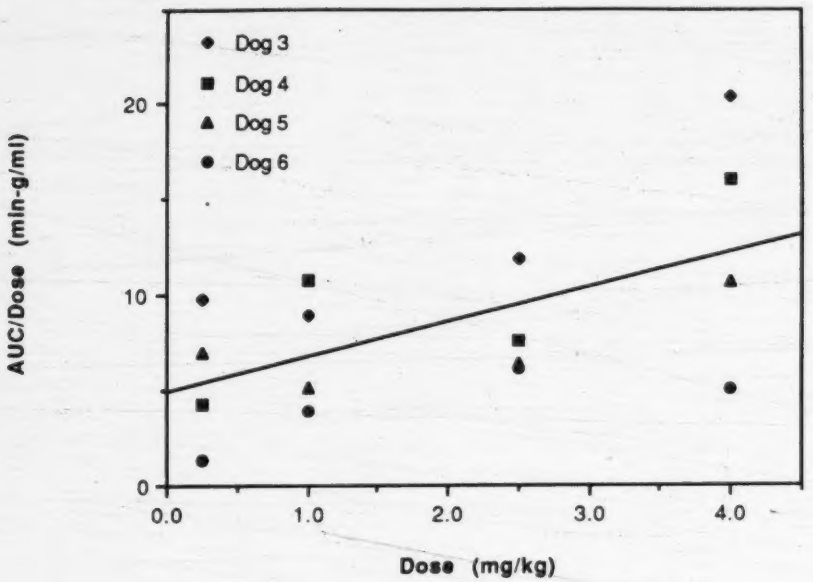


Figure 5-9. Dose-corrected AUC *vs.* dose with best-fit simple regression line for the four subjects. ($y = 4.9 + 1.8x$, $r^2 = 0.319$)

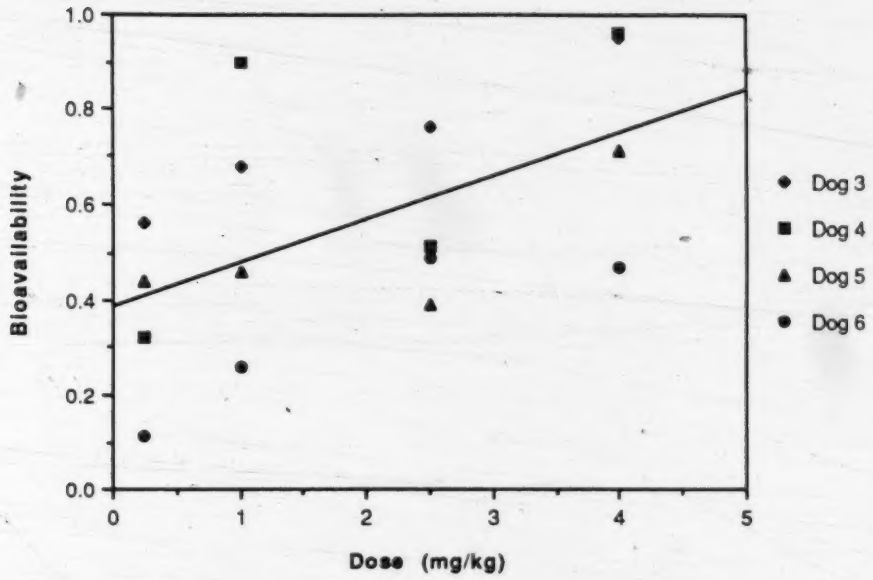


Figure 5-10. F *vs.* dose with best-fit simple regression line for the four subjects. ($y$ = $0.38 + 0.091x$, $r^2 = 0.319$)

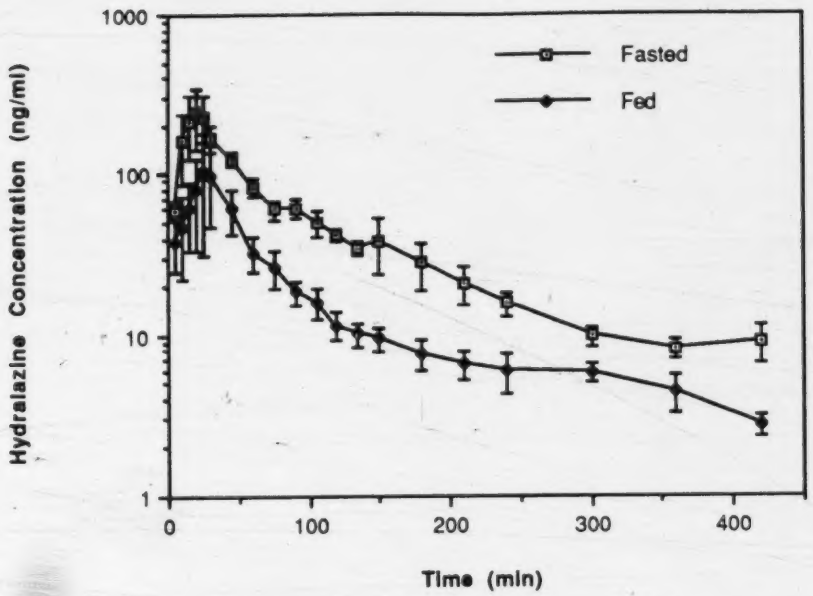


Figure 5-11. Log mean (± SE) hydralazine concentration *vs.* time after 2.5 mg/kg oral doses of hydralazine·HCl liquid in fasted or fed dogs.

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# 6. Physiological Modelling in the Conscious Dog: Mechanisms of the Food Effect on Hydralazine Kinetics

## 6.1 Introduction

Although the interactions between food and drugs that undergo extensive first pass metabolism have been studied for over a decade, little is known about the hepatic mechanisms involved. One reason may be that the physiological models used to describe the food effect have never been tested directly. Indirect methods have ruled out the transient increase in hepatic blood flow after a meal as being the only mechanism responsible for the increase in the bioavailability of propranolol. Studies on anesthetized animal models using methods to mimic aspects of the food effect while measuring inlet and outlet concentrations and hepatic blood flow have yielded equivocal results (1, 2). It would be helpful to measure these parameters in the conscious animal in the fasted and fed state so that a direct comparison could be made.

A surgical technique has been developed wherein dogs can be implanted with four catheters (portal vein, hepatic vein, carotid artery and jugular vein) and two flow probes (portal vein and hepatic artery) in a chronic preparation which has a useful life of 6-8 weeks. Using blood sampling from these catheters and continuous flow monitoring from the flow probes, all the parameters of a physiological model of the liver can be measured simultaneously in the conscious animal (Figure 6-1). This model has been applied first to a study of the food effect on hydralazine kinetics.

## 6.2 Methods

### 6.2.1 Animal Model Preparation

#### 6.2.1.1 Implant Preparation

Four silastic catheters, 0.062 in id x 0.125 in od (Lengths: Carotid, 70 cm, Jugular, 70 cm, Hepatic, 80 cm and Portal, 70 cm) (Dow Corning, Midland, Michigan) and two ultrasonic transit time flow probes (Transonic, Ithaca, New York), 4 mm for the hepatic artery and and 8 mm for the portal vein, were cleaned with chlorhexidine scrub (Hibitaine) and rinsed with distilled water. Double velour dacron (Meadox, Oakland, New Jersey) cuffs were placed 15 cm from the external ends of all the devices. The dacron velour was cut into 2 cm diameter discs and a 2 mm hole made in each disc. Two such discs were then threaded onto a catheter or flow probe lead. Between the discs and 15 cm from one end of the catheter, a bolus of medical grade silicone elastomer glue (Silastic, Dow Corning, Midland, Michigan) was applied around the catheter and to the two discs which were then squeezed together to form a laminate held together and to the catheter by the glue (Figure 6-2). At the edge of the disc, the glue thickness was 1 mm and towards the middle, about 3-4 mm. To provide an anchor point in the vessel, each catheter was given a 4 mm diameter Silastic glue 'button' near the end of the catheter opposite to the disc (Figure 6-2). The button positions, in cm from the end to be implanted in the vessel were, carotid and jugular, 15-20, hepatic and portal, 10. The preparations were allowed to dry for 24 h before being packaged and sent for ethylene oxide gas sterilization.

Some of the flow probes had pin connectors, which were unsoldered from their leads before preparation, and some were fitted with Konigsberg skin buttons. The Konigsberg buttons were not prepared as above, but dacron velour discs, the same

diameter as the button, were cut and glued to the flat surfaces with cyanoacrylate or Silastic glue. To fit the connector portion of the skin buttons, a 4 mm hole was cut in the dacron disc for the top surface using a disposable skin biopsy punch. They were also gas sterilized before use.

Probe reflectors, screws and a screw driver were sterilized separately because they were opened during Stage II of the surgery.

#### 6.2.1.2 Surgery

##### 6.2.1.2.1 Anesthesia and Preparation

Eight random source, intact male, mixed breed dogs weighing 18-23.5 kg (mean 22 kg) were selected for the study. After an overnight fast, each dog was administered a preanesthetic mixture consisting of acepromazine, meperidine and atropine. When the animal was sedated, a halothane/$O_2$ mixture was administered by mask until a surgical plane of anesthesia was reached. The subject was then intubated, clipped and prepared for sterile surgery. The area clipped consisted of the back, neck, right side and abdomen.

##### 6.2.1.2.2 Stage I: Subcutaneous Placement of Catheters

###### 6.2.1.2.2.1 Preparation of Skin Interface Sites and Subcutaneous Pockets

The subject was first placed in sternal recumbency with the head and hind limbs turned to the right to expose the right aspect of the neck and the right flank. After preparation for surgery and draping, a dorsal midline incision was made from the cranial point of the shoulder caudally for about 18 cm. A row of six subcutaneous pockets was then made by blunt dissection, extending about 4 cm to the right of the incision. Another 3 cm incision was made in the neck cranial to the point of the shoulder and a pocket large enough to hold two catheters formed in a ventral direction

towards the jugular vein by blunt dissection. A third 3 cm incision was made vertically in the right flank about 8-10 cm caudal to the last rib. A pocket extending cranially to cover the costovertebral angle was formed by blunt dissection. It was made large enough to hold two catheters and the two flow probes.

6.2.1.2.2.2 Placement of Catheters

Intestinal forceps were then passed bluntly from the neck pocket through the edge of the cranialmost pocket on the back. The carotid catheter was grasped and pulled through into the neck pocket until the dacron flange lay in the dorsal pocket. A small (1-2 mm) stab incision was made in the middle of the pocket about 3-4 cm from the midline incision using a #11 scalpel blade and the external end of the catheter pulled through using mosquito hemostatic forceps, until the flange lay snug against the subcutis. This process was repeated with the jugular catheter in the second pocket and the vessel ends of both catheters were coiled and placed in the neck pocket. The neck pocket was closed with two subcutaneous layers of simple continuous 3-0 Dexon sutures and the skin closed with simple interrupted 3-0 stainless steel sutures. The same process was repeated with the four remaining catheters and flow probes in the flank pocket. Tunnelling was accomplished using 30 cm De Lee dressing forceps. If the flow probes were fitted with Konigsberg skin buttons, 3-4 mm holes instead of stab incisions were made at the skin exit points to accommodate the pin connectors.

6.2.1.2.2.3 Skin Closure

The first suture layer of the dorsal midline incision was 3-0 Dexon, placed close to the velour flanges to eliminate dead space. A second simple continuous subcutaneous layer of 3-0 Dexon was followed by simple interrupted 3-0 stainless steel skin sutures.

6.2.1.2.2.4 Sealing of External Ends of Catheters

Teflon intravenous placement catheters (14 ga x 2 in) were then cut to about 2.5 cm and inserted to the hilt into the open ends of the catheters. They were glued in place using cyanoacrylate glue. Male luer lock caps with rubber septa (PRN adaptor, Deseret Medical, Inc., Sandy, Utah) were used to seal the ends of the catheters. The sealed catheters were then filled with Anticoagulant-citrate-dextrose (ACD) Solution B, U.S.P. with 1.5% formaldehyde added to sterilize the catheter lumens. The drapes were then removed and the subject turned over, prepared and draped for stage II of the surgery. At this time, about 1.5 h had elapsed since the subject was anesthetized.

### 6.2.1.2.3 Stage II: Intra-abdominal Placement of Catheters

6.2.1.2.3.1 Approach to the Abdomen

An extensive ventral midline incision was made from the xiphoid to the prepuce. Bleeders were cauterized. The duodenum was retracted to expose the portal vein area and right flank. The small intestine was packed off using a moist laparotomy sponge.

6.2.1.2.3.2 Catheter Retrieval

The location of the right flank pocket containing the catheters and flow probes was palpated at the costovertebral angle next to the right kidney. After confirmation of the location, a blunt incision through the abdominal wall into the pocket was made with Metzenbaum scissors. A Love nerve retractor was used to retrieve the catheters and flow probes. Angled peripheral vascular Debakey forceps were then used to puncture the membrane which attaches the caudal vena cava to the dorsal wall of the abdomen, just cranial to the cranial pole of the right kidney. The two flow probes and the portal vein catheter were passed through this hole beneath the vena cava to lie near the portal vein. In this position, the catheters could not interfere with the small intestine.

6.2.1.2.3.3 Hepatic Arterial Flow Probe Placement

The hepatic arterial flow probe was placed first. The hepatic artery is a major branch of the celiac artery , and is visible as it courses cranially next to the caudal vena cava for a distance of about 6 cm. It is covered by a nerve net. To place the probe, the artery was freed by blunt dissection, usually along a reasonably straight portion where there was a gap in the nerve net. The artery was lifted with Mixter forceps and the reflector of the probe placed under the vessel (The probe lead exited caudally). The retainer plate was then bolted into place. A stay suture (3-0 silk) was placed to fasten the flow probe to the wall of the vena cava and a piece of neighboring tissue in such a way that the artery passed unobstructed through the probe lumen. A second stay suture attached the probe lead to the vena cava (Figure 6-2).

6.2.1.2.3.4 Portal Venous Flow Probe Placement

The portal venous probe was placed next. The entrance of the gastroduodenal vein into the portal vein was located and a 2 cm section of portal vein caudal to this was freed by gentle blunt dissection. The freed section of portal vein was elevated using Mixter forceps and the 8 mm probe positioned with the probe lead exiting caudally. The retainer plate was bolted in place and the probe and lead were anchored to the surrounding tissue with 2-0 silk sutures so that the probe did not obstruct blood flow. Protruding pieces of fatty tissue were trimmed from around the probe. This was necessary because fat has poor acoustical conduction and could lead to instrumental measurement errors if it were allowed to enter the lumen of the probe.

6.2.1.2.3.5 Gastroduodenal Artery Ligation

The hepatic artery sends branches to each liver lobe, then continues distally as the gastroduodenal artery. To eliminate extrahepatic blood flow, this branch was

ligated. The regions of the stomach, pancreas and duodenum supplied by the gastroduodenal artery have a collateral blood supply which compensates adequately following its ligation.

6.2.1.2.3.6 Portal Vein Catheter Placement

The gastroduodenal vein empties into the portal vein about 1.5 cm from the hilus of the liver, downstream from the flow probe. This vessel was utilized as an entry point for the portal vein catheter. About 1-2 cm from the portal vein the gastroduodenal vein emerges from the body of the pancreas. At this position, it was freed by blunt dissection, ligated upstream and a loose ligature was placed downstream, close to the entry to the portal vein. back flow from the portal vein was prevented by putting tension on the downstream ligature. A small cut was made in the wall of the vessel and the portal vein catheter was inserted and fed downstream into the portal vein. It was very important to palpate the position of the catheter because it tended to pass across the portal vein and become lodged in the portal branch supplying the caudate process and right lateral lobe. In this position, the catheter was extremely prone to blockage. Therefore, it was positioned ideally by palpation, then removed and trimmed if necessary so that the tip lay in the hilus when the catheter was inserted fully to the anchor point. The ligature was gently tightened, tied and then tied again above the anchor point on the catheter. The gastroduodenal vein enters the portal vein almost at a right angle. In order to direct the catheter more in the direction of flow in the portal vein and to prevent blockage of the catheter, the following manipulation was performed. A second ligature was placed above the catheter on the gastroduodenal vein. The vein was then transected between the ligatures and the catheter moved in line with the portal vein. The catheter was fastened to the portal flow probe to stabilize its position, and the position was checked by palpation. The duodenum was replaced in position.

6.2.1.2.3.7 Hepatic Vein Catheter Placement

To install the hepatic vein catheter, the left lateral lobe of the liver was grasped using a wet sponge and drawn into the field of view. The diaphragmatic surface was palpated to detect a depression where a large branch of the hepatic vein ran through the parenchyma. This depression could usually be palpated along its length to the common hepatic vein, although the intrasubject variation in anatomy was large. At the distal end of the depression, an incision was made in the liver capsule and with the end of a scalpel handle, the liver parenchyma was bluntly dissected to expose the vein. The inevitable oozing of blood from the parenchyma was minimized by applying pressure from beneath the lobe. A stay suture was placed through the exposed wall of the vein to anchor the catheter. No other tissue in the liver is strong enough to use as an anchor. Just proximal to the stay suture, the vein was nicked and the catheter introduced. Since it is possible in some animals to mistake a branch of the portal vein for the hepatic vein (this occurred on two occasions), two methods for confirming the placement of the catheter were used. First, the catheter ends were made longer so that they could be inserted well into the vena cava and palpated at the intersection of the vena cava and diaphragm. Secondly, the back pressure in the vein was checked by evaluating leakage when manual pressure on the vessel was removed. A portal branch would quickly flood the site with blood, whereas the lower pressure hepatic vein would leak very little. After confirming that the vein was indeed a branch of the hepatic vein, the catheter was trimmed so that when the anchor point abutted the entry point into the vein, the tip of the catheter lay about 1 cm into the common hepatic vein.The stay suture was then fastened above the anchor point on the catheter. The capsule was closed with 3 horizontal mattress sutures to prevent bleeding. The catheter was anchored to the body wall to the right of the xiphoid with a stay suture. Extra lengths of catheter and flow

probe lead were gathered in coils along the right body wall and fastened there with stay sutures to prevent interference with the intestines.

6.2.1.2.3.8 Ensuring Catheter Patency

Before closure of the abdomen, catheter patency was checked. The ACD solution was removed and the catheters were flushed with sterile saline, followed by a 1,000 U/ml heparin lock. About 0.1 ml more heparin solution than the dead space of the catheters was used. Use of a large excess was avoided to prevent bleeding.

6.2.1.2.3.9 Abdomen Closure

The linea alba was closed with simple interrupted 0 Dexon sutures, the subcutaneous layer was simple continuous 3-0 Dexon and the skin was closed with simple interrupted 3-0 stainless steel sutures.

6.2.1.2.3.10 Placement of Carotid Arterial Catheter

To place the jugular and carotid catheters, a 4 cm ventral midline incision was made in the caudal portion of the neck. The subcutaneous fascia was bluntly dissected to expose the pocket containing the catheters. A hole was made in the pocket and the catheters were retrieved. The neck muscles were separated down to the trachea and the loose fascia was separated to expose the right external carotid artery. The artery was exposed, stripped and clamped at least 2 cm upstream from the catheterization site. A 2-0 silk ligature was placed downstream from the catheterization point and used to elevate the vessel. Two loose ligatures were placed around the vessel near the clamp. A small incision was made in the wall of the artery, and the catheter was introduced through the incision and advanced to the level of the clamp. One of the loose ligatures was tightened around the artery to prevent leakage of blood around the catheter as it was fed towards the heart. The clamp was then removed and the catheter was fed into the artery until the

anchor point on the catheter was reached. The catheter was doubly ligated in place and the downstream ligature was fastened above the anchor point on the catheter. The artery was then replaced in position and if necessary, stay sutures were placed to prevent kinking of the catheter. The neck muscles were apposed with simple continuous 3-0 Dexon sutures.

6.2.1.2.3.11 Placement of Jugular Venous Catheter

The right external jugular vein was exposed by blunt dissection and stripped for a 3 cm length. It was ligated with 2-0 silk at the upstream end of the exposed section and a loose ligature was placed downstream. An incision was made in the wall of the vein, and the catheter was introduced and fed to the anchor point. The loose ligature was tightened and tied, and the upstream ligature was fastened above the anchor point. The vessel was replaced and the extra catheter material coiled beneath the skin so that it could not kink. The dead space was closed with 3-0 Dexon sutures, and the subcutaneous and skin layers were closed as for the abdomen. The total surgery time for stages I and II was about 4 h.

**6.2.1.2.4 Recovery and Aftercare**

The anesthetic was turned off and the animal allowed to recover. During recovery, the flow probe leads were re-soldered to their respective connectors and the flow probe connections were checked. Acoustic errors were often initially encountered, presumably due to air spaces between the vessels and probes, but these disappeared in a few days as the probes healed in place. The dogs were fitted with jackets with pockets (Alice King Chatham Medical Arts, Los Angeles, California) to protect the catheters and flow probe leads.

Upon waking, the animals were allowed to recover in heated cages and were given buprenorphine for analgesia as required. The day following surgery, blood was

taken for a complete blood count, serum protein, liver enzymes, and serum amylase. The preparation was allowed to heal for at least ten days before experiments were started.

#### 6.2.1.3 Catheter Care

Dilute gentamicin solution was injected around the skin interfaces twice daily when oozing was encountered. The catheters were flushed every 3-4 days by cleaning the PRN adaptors with disinfectant, removing the heparin locks, instilling saline, then ACD solution with formaldehyde to fill the catheter dead space. The ACD was left for 5 min to sterilize the catheter lumens and then removed. The catheters were flushed with sterile saline and then filled with heparin solution. After 1 week of healing, the heparin strength was increased from 1,000 U/ml to 10,000 U/ml. The PRN adaptors were changed regularly as required. A sling was used for restraint during the procedures. After the surgery, the subjects were fed canned dog food only, to adapt them to the test diet.

#### 6.2.1.4 Complications

Problems encountered included blocked catheters, skin interface infections, septicemia, and chewing of catheters and probe leads. Blocked catheters soon after the surgery were invariably due to mechanical blockage. They could often be freed by manipulating the position of the subject, but occasionally if simply left until another day, they would spontaneously become free. Blockages later in the life of the preparation (usually after four weeks post-surgery, unless an episode of septicemia had occurred) were more serious, because they were due to growth of a fibrous sheath around the catheter. Fluid could be infused but not withdrawn, because the sheath would collapse around the catheter tip, blocking it. This was confirmed on postmortem examination. Such a blockage meant termination of experiments on that subject. Skin

interface infections became rare as experience was gained. If they did occur, they were usually localized. Occasionally, an animal would chew through the jacket and damage the catheters and flow probe leads. The flow probe leads were spliced and re-soldered and the catheters ends were trimmed and refitted with teflon catheters and PRN adaptors. The most serious consequence of chewing was that bacteria could be introduced directly into the bloodstream via the damaged catheter to cause a septicemia. If this occurred, blood cultures were performed and appropriate antibiotics administered on the basis of culture results (penicillin, cloxacillin or gentamicin, because they are excreted mostly unchanged in the urine). After recovery, the subject was not used for an experiment for at least one week after the antibiotic treatment had finished.

### 6.2.2 Pharmacokinetic Studies

On the day of an experiment, the subject was brought to the laboratory early in the morning, having been fasted overnight, and placed in a sling frame. The flow probe leads were attached to the meter and flow recording was started. The PRN adaptors were removed from the catheter ends and replaced with minimum volume extension sets (Cutter Biological, Elkhart, Indiana). Three way stopcocks were attached to the ends of the extension sets and the heparin was removed from the catheters and replaced with 10 U/ml heparin in sterile saline. While the dog was being prepared by one team member, other members set up the equipment for the hydralazine assay and prepared 1 ml tuberculin syringes for blood sampling by filling the hubs with 0.1 M EDTA, as anticoagulant. A 20 ml blank blood sample with 0.5 ml of 0.1 M Na EDTA was taken from the jugular vein catheter for the standard curve. The dose of hydralazine was prepared by drawing 50 ml of 0.01 N HCl into a 60 ml syringe. The calculated dose of hydralazine HCl (2.5 mg/kg of 20 mg/ml Apresoline® solution) was drawn into a 3 ml syringe and injected into the 60 ml syringe. The solution was thoroughly mixed.

Before the drug was administered to the subject, blank blood samples were drawn simultaneously from the 4 catheters by the following method. The 3- way stopcocks were each outfitted with a 10 ml syringe and a 1 ml sample syringe. Starting 30 sec before the blood sampling time, fresh blood was drawn into the 10 ml syringes at a rate of about 1 ml/5 sec. At 5 sec before the sample was to be taken, the stopcocks were switched to the sampling syringes and exactly 1 ml of blood was drawn. The sample syringes were then removed and emptied into test tubes for further processing according to the hydralazine assay procedure. The blood from the 10 ml syringes was injected back into the catheters and flushed with about 2 ml of 10 U/ml heparin in saline to form a light heparin lock.

The hydralazine dose was administered by tilting back the animal's head, pulling on the caudal portion of the lip to form a cheek pocket and slowly emptying the dose syringe into the pocket. If necessary, the glottal region of the throat was massaged to encourage swallowing. This process usually took between 1-2 min. When the last of the dose had been swallowed, the clocks were started from time 0.

Blood samples were taken simultaneously from all 4 catheters by the same method as for the blank samples at 2.5, 5, 7.5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min. The catheters were not flushed with heparin between the start and 10 min due to time limitations but the blood in the 10 ml syringes was always injected back into the catheters between samples. The total volume of blood sampled per experiment was less than 100 ml. At the termination of the experiment, the flow probe leads were disconnected, and the catheters were supplied with new PRN adaptors, sterilized with ACD solution and given 10,000 U/ml heparin locks. The dog was returned to its cage and fed.

"Fed" experiments were conducted in similar fashion, except that immediately before the drug dose was given, each subject received 723 g of commercial canned dog

food (Dr. Ballard's brand, beef flavor). This was consumed in less than 2 min in every case.

During experiments, the animals usually were somewhat active in the sling for the first two h, but most spent some time sleeping after fatigue from standing set in. When an animal became very agitated in the middle of an experiment, it was disconnected, removed from the sling and allowed to urinate and defecate. It was then replaced in the sling, reconnected, and the experiment was continued.

A minimum 3 day washout period was allowed between experiments.

### 6.2.3 Sample Analysis

The samples were derivatized with p-nitrobenzaldehyde and analyzed by HPLC as previously described (Chapter 3, Chapter 5). Since 4 samples were collected simultaneously, the samples were processed in quick succession by a team of individuals who added internal standard and derivatizing reagent, then vortexed them. The entire process could be completed in less than 1 min.

### 6.2.4 Pharmacokinetic Analysis

It was assumed that the carotid arterial concentration was the same as the hepatic arterial concentration. Based on the observation that the post-absorption concentration of hydralazine in the portal vein was almost always greater than or equal to the concentration in the carotid artery, it was also assumed that gastrointestinal extraction was negligible. The absorption rate was calculated at each time point from influx, which is $(C_{pv}-C_{ca})*Q_{pv}$. These data were then integrated to give the total amount absorbed to 480 min (3). The fraction absorbed was calculated by dividing the amount absorbed by the dose of hydralazine base given.

Extraction ratios were calculated at each time point as:

$$E = (C_{ca}*Q_{ha}+C_{pv}*Q_{pv}-C_{hv}*(Q_{ha}+Q_{pv}))/ (C_{ca}*Q_{ha}+C_{pv}*Q_{pv})$$

Overall hepatic extraction was calculated by:

$E$ = (Total input - Total output) / Total input

Total input = Area under the hepatic influx *vs.* time curve

Hepatic influx = $C_{ca}*Q_{ha}+C_{pv}*Q_{pv}$

Total output = Area under the hepatic efflux *vs.* time curve

Hepatic efflux = $C_{hv}*(Q_{ha}+Q_{pv})$

Cumulative extraction was calculated similarly to total extraction; for every sampling time, the cumulative areas under the flux *vs.* time curves were substituted for the total areas (3).

Hepatic Clearance was calculated at each time point as:

$Cl_h = (Q_{ha}+Q_{pv})*E$

Hepatic intrinsic clearance ($Cl_{int}$) was calculated at each time point by two methods, according to the venous equilibrium ("well-stirred", WS) model and according to the sinusoidal perfusion model ("parallel tube", PT), which represent the boundary conditions for modelling hepatic removal of substances passing through the liver (4):

$Cl_{int} = E*Q/(1-E)$ (WS)

$Cl_{int} = -Q*\ln(1-E)$ (PT)

The mean (± SD) of all data points after 10 min was used as the $Cl_{int}$ for each dog.

Jugular venous concentration *vs.* time data and absorption were analyzed by non-compartmental methods (5). The computer program LAGRAN was used to integrate the concentration *vs.* time curves and calculate the pharmacokinetic parameters (3). In addition, by integrating fluxes in the various vessels *vs.* time using the same program, cumulative amounts of drug absorbed and extracted could be calculated as described above.

### 6.2.5 Statistical Analysis

A randomized crossover design was used for the pharmacokinetic studies. Comparisons between peripheral blood parameters from the fasted and fed states were made by ANOVA (6), with $p = 0.05$. Comparisons of physiological model data between the fasted and fed states were made by paired t-tests with $p = 0.05$.

## 6.3 Results

### 6.3.1 Portal Venous Mixing

In two dogs, catheters were placed in two locations in the portal system, the hilus of the liver and about 4 cm downstream in the left lateral lobe. Five sets of concentration *vs.* time data were gathered, 2 from one dog and 3 from the other; resulting in a total of 88 pairs of portal venous data. The mean difference in hydralazine concentration between the two portal venous sampling points was 4.0% ± 16 % for the 88 sample pairs. A histogram of the distribution of mean differences is shown in Figure 6-3. It shows a slightly positively skewed normal distribution. These data demonstrate that the overall effect of incomplete mixing in the portal vein would be slight, and that the samples taken from the main portal catheter are representative of the concentration being delivered to the lobe of interest.

### 6.3.2 Initial Sample

The first event of note occurs at the very first sample taken. Using both the fasted and fed data from six dogs, of the 10 full sets of 2.5 min samples (2 sets contained missing values for one or more vessels), 9 have the lowest concentration of hydralazine in the hepatic vein, the sequence of concentrations being, from high to low, Portal:Jugular:Carotid:Hepatic. These data are not consistent with the assumption that all of the orally administered drug is absorbed from the gastrointestinal tract and

delivered to the liver. The presence of higher concentrations of drug in the systemic circulation than in the hepatic vein so soon after dosing indicates pregastric absorption. Since the jugular venous concentrations were slightly, but consistently higher than the carotid arterial concentrations at this time, the most likely initial site of pregastric absorption is the oral cavity, the blood supply of which is drained by the jugular vein. Secondly, the very low initial concentrations in the hepatic vein, as compared with the portal vein, indicate that there is a significant lag time before large amounts of hydralazine begin to leave the liver.

After the first sample, in all cases, carotid arterial concentration rose very quickly to exceed jugular venous levels, and in some cases exceeded even hepatic venous concentration (See Figure 6-4). This rapid increase was undoubtedly partially due to a contribution by the hepatic vein. After drug leaves the hepatic vein, it passes through the caudal vena to the heart, without being exposed to the jugular catheter. The carotid artery would therefore be exposed to drug originating from the liver first, before it had distributed to the rest of the body, while at least some dilution and distribution could have occurred by the time blood had circulated back to the jugular vein. In addition, drug absorbed through the esophagus would drain into the azygous vein. In the dog, the azygous vein empties into the systemic circulation at the entrance to the right atrium, downstream from both the jugular venous and hepatic venous catheters. Drug absorbed from the esophagus would be detected first in the carotid artery catheter.

### 6.3.3 Absorption

The amount of hydralazine absorbed was calculated to be 12.4 ± 6.8 mg for the fasted condition and 9.0 ± 3.1 mg for the fed condition, comprising 28 ± 15% and 20 ± 7% of the dose given, respectively (Table 6-1). These values were not significantly different ($p > 0.05$). Peak absorption rates occurred at 5.7 ± 2.6 min and 8.1 ± 3.5 min

(not significantly different, $p > 0.05$), for the fasted and fed conditions, respectively. Maximum influx coincided very closely with $t_{max}$ (PV). Although the mean peak absorption rate for the fed state ($0.58 \pm 0.44$ mg/min) was less than half that for the fasted state ($1.38 \pm 1.21$ mg/min), this was not significant due to the variability of this parameter. In fact, peak absorption rates in three of eight dogs were greater in the fasted than in the fed state. One indicator of absorption, however, was significantly different between the fasted and fed states. Portal venous $C_{max}$ decreased 51% from $2931 \pm 1311$ (fasted) to $1438 \pm 901$ ng/ml (fed, Table 6-4).

### 6.3.4 Hepatic Parameters

#### 6.3.4.1 Hepatic Blood Flow

Hepatic arterial blood flow was unaffected by food. In both groups, within 20-25 min, $Q_{ha}$ peaked to approximately 3 times the flow measured at the first sampling time, from 100 to almost 300 ml/min, then gradually returned to initial levels about 2 h after dosing (Figure 6-5). On the other hand, portal venous blood flow was significantly affected by food. Mean $Q_{pv}$ levels initially increased from about 350 to 500 ml/min during the first few minutes but returned to baseline values after only about 1 h post-dosing in the fasted condition (Figure 6-6). In the fed condition, the initial increase in mean portal venous blood flow was greater, from 350 to 600 ml/min, and after 1 h, declined to a level elevated from initial values, about 500 ml/min, where it remained for the duration of the experiment (8 h). Total hepatic blood flow followed a similar pattern to portal venous blood flow (Figure 6-7). When the results were pooled, paired t-tests showed significant differences between the fasted and fed conditions for portal venous blood flow and total blood flow, but not for hepatic arterial blood flow. Point by point comparisons of flow data could not distinguish any times when the differences were particularly prominent, due to the small sample sizes and high

variability of the individual blood flow rates. Figures 6-6 and 6-7, showing mean portal venous and total blood flow *vs.* time, indicate that the differences between the groups were fairly constant from about 1 h to the end of the experiments.

#### 6.3.4.2 Extraction Ratio

Point by point t-tests did not reveal any time when E was significantly different between the nutritional states due to high variability in the data and perhaps mismatches in times at which related events, such as peak concentration and blood flow, occurred, so the analyses are based on cumulative and total extraction calculated from integration of flux data (Table 6-1).

Initially, hepatic extraction is high due to distribution and elimination at the same time in the liver. Extraction decreases over time as these processes equilibrate. This reduction in extraction would be difficult to distinguish from a decrease in extraction caused by transient saturation of hepatic metabolism if they occurred simultaneously. If cumulative hepatic extraction was plotted *vs.* time, as hepatic efflux increased, cumulative extraction would approach an asymptote, total hepatic extraction. If decreased hepatic extraction due to transient saturation of metabolism continued past the time when the equilibration processes were dominant, however, cumulative extraction could transiently dip below total extraction. Since this could not occur with only equilibration processes, a transient drop in cumulative extraction to values below total extraction can be interpreted as evidence for saturation of hepatic metabolism. The total cumulative E of hydralazine did not change significantly between the fasted and fed conditions. For the 2 subjects with the highest fasted extraction, the extraction ratio decreased slightly in the fed condition (0.69 to 0.62 and 0.66 to 0.60), but in the other 4 dogs extraction was increased in the fed condition. Two dogs had very low extraction ratios in the fasted condition (0.18); these were the only subjects which showed

evidence of saturation of metabolism. In these animals, cumulative E dropped below total E early in the cumulative extraction vs. time profile (see Figure 6-8). Saturation of hepatic metabolism was not observed in any subject in the fed state; in both animals showing saturation of metabolism in the fasted state large increases in hydralazine extraction were observed when they were fed (from 0.18 to 0.51 and 0.55).

Extraction by the lung or gastrointestinal tract is unlikely because after about 2 h, when absorption was virtually complete, jugular venous and carotid arterial concentrations of hydralazine were almost identical and portal venous concentrations almost never fell below carotid arterial concentrations.

#### 6.3.4.3 Clearance

Accurate measurement of initial clearance is hampered by the same processes of distribution and metabolism which affect extraction. A cumulative measure of clearance is not possible, however. Instead, the clearances for each dog were averaged over 15 - 480 min to give mean clearance values which were used in the comparisons between the fasted and fed conditions (The clearance values at 2.5-10 min were not used in the calculations because they appeared to be significantly influenced by equilibration phenomena in the liver). The method is biased in that it is weighted toward values at times when little of the drug is being extracted. This is overcome somewhat by the timing sequence of the samples; more frequent samples were taken early in the experiments. A method of calculating mean clearance using weighting according to a parameter such as the amount of drug cleared or extracted by the liver over an interval would be more valid, but this presents more serious problems because samples were available only at the beginning and end of each time interval. Weighting according to this method would require estimates of clearance from data collected at the mid-point of an interval.

Mean hepatic clearance increased over 50% after feeding, from 11 ± 4 ml/min/kg to 17 ± 7 ml/min/kg

#### 6.3.4.4 Intrinsic Clearance

Because cumulative E did not change significantly after 10 min, it was assumed that linear kinetics were operating in the concentration range observed. Therefore, the equations developed by Pang and Rowland (4) could be used to calculate $Cl_{int}$. Estimations of intrinsic clearance were made according to both the "Well Stirred" (WS) and "Parallel Tube" (PT) models and qualitatively similar results were obtained (Table 6-2). In the fasted state, mean $Cl_{int}$ was 24 ± 13 ml/min/kg (WS) or 15 ± 7 ml/min/kg (PT). In the fed state, the means were significantly higher ($p < 0.05$), 38 ± 22 ml/min/kg (WS) or 24 ± 11 ml/min/kg (PT), for an increase according to both models of 60%.

### 6.3.5 Systemic Parameters

The results are summarized in the Table 6-3. Jugular venous hydralazine concentrations were assumed to be representative of systemic blood concentrations (Table 6-4). In all cases, results from analysis by ANOVA of the crossover design agreed with those from paired t-tests. Indicators of bioavailability, including AUC and jugular $C_{max}$, decreased 44% and 52%, respectively from the fasted to the fed state ($p < 0.05$ in both cases). $C_{max}$ was also reduced by 51% in the portal vein, 64% in the hepatic vein and 64% in the carotid artery ($p < 0.05$, Table 6-4). No significant changes in hydralazine $t_{max}$ were observed for any of the vessels.

$\beta$ was not significantly increased (0.0048 ± 0.0011, fasted and 0.0067 ± 0.0027 $min^{-1}$, fed). Mean $t_{1/2}$ dropped from 151 ± 36 min to 113 ± 31 min (Arithmetic means). Mean residence times did not change significantly with feeding (107 ± 31, fasted to 118 ± 29 min, fed).

## 6.4 Discussion

### 6.4.1 Portal Venous Concentrations

The short length of the portal vein gives rise to the question of whether complete mixing of drug has occurred before the drug has reached the portal venous blood sampling point at the hilus of the liver. If mixing is incomplete, then systematic errors in the estimation of drug concentrations entering the lobe of the liver from which data is being gathered could occur, leading to errors in pharmacokinetic parameter estimation. In two dogs, during the surgical preparation, catheters intended for the hepatic vein were inadvertently installed into an intralobar branch of the portal vein. Thus the portal system was catheterized twice, with one catheter approximately 5 cm downstream from the other. This provided a means of estimating whether incomplete mixing or streaming in the portal vein could cause systematic errors in the estimation of extraction. The concentrations of hydralazine in blood drawn from the two catheters were compared on a point by point basis. Concentrations ranged over 4 orders of magnitude. Differences in concentration were calculated and expressed as a percent of the concentration measured in the catheter placed at the hilus of the liver (Figure 6-3).

The good agreement between portal venous concentration data gathered from the two sites supports the assumption that the sampling site at the hilus of the liver gives a reliable measurement of portal hydralazine concentration.

### 6.4.2 Absorption

Despite reports in the literature indicating that radiolabelled hydralazine is rapidly and completely absorbed after oral administration, the calculated amount

absorbed based on serial portal venous sampling was less than 30% of the dose of hydralazine given. The radiolabel studies carry the limitation that they cannot distinguish between hydralazine and metabolites or decomposition products, and they cannot distinguish between portally and systemically absorbed drug. The bioavailability of hydralazine in the dog, as calculated by the AUC method, was about 54 ± 8% at 2.5 mg/kg (Chapter 5), and the fasted oral AUC's from this study agree well with those from the previous study (21839 ± 4885 ng-min/ml *vs.* 19923 ± 3364 ng-min/ml, respectively). Yet the systemic availability calculated from hydralazine fluxes in this study was only 13 ± 5%. The discrepancy is too large to be accounted for only by a series of inaccuracies in measured parameters such as blood flow and hydralazine concentration. The blood flows observed in the fasted condition were very similar to those observed by Heinzow *et al.* (7). In addition, these subjects were used in studies of hepatic insulin extraction, and blood flows in both vessels were consistent with previous studies wherein other methods of flow measurement were used. Hydralazine concentrations were determined from standard curves based on freshly prepared authentic standard compounds, and the concentrations of drug in the vials from which the doses were taken were measured during each experiment and found to be close to the labelled concentration.

The data were therefore accepted as accurate and the assumptions of the indirect method of estimating F, namely the AUC method, were examined. A major assumption of this method is that all orally administered drug is absorbed into the portal circulation and must pass through the liver before reaching the systemic circulation. This assumption may not be valid, given the order of concentrations seen in most cases in the first sample taken. Since larger concentrations of hydralazine were usually present in the systemic vessels than in the hepatic vein, the possibility of some hydralazine being absorbed outside the portion of the intestinal tract drained by the portal vein must

be examined. Pregastrically absorbed hydralazine would add to the AUC of hydralazine, not be metabolized on the first pass, and would not be included in the portal venous concentration. Calculation of absorption based only on portal influx would therefore lead to an underestimation of the fraction of the dose absorbed. Additional evidence for pregastric absorption lies in the total amount of hydralazine extracted by the liver (Table 6-1). It exceeded the total amount absorbed through the portal vein in several instances, even though hepatic clearance (mean fasted ~ 11 ml/min/kg) is only about 16% of the mean total body clearance calculated for the four dogs in Chapter 5 (68 ml/min/kg). The evidence for at least some pregastric absorption led to the hypothesis that a large portion of the dose could have been absorbed from the mouth and esophagus into the systemic circulation. To test whether this hypothesis was consistent with the concentration *vs.* time data in the portal vein, hepatic artery and hepatic vein, a series of computer simulations was performed (Appendix 1). A schematic diagram of the model is depicted in Figure 6-9. The results of the simulations are shown in Figures 6-10 to 6-13. The 2.5 mg/kg intravenous data from the previous study were well fit by the model after a mixing period and until after 90 min, which comprised most of the AUC (Figure 6-10). After 90 min, the concentration simulated by the model declined more rapidly than the observed concentrations, possibly due to draining of a deep tissue compartment not accounted for by the model. This is not a serious deficiency because only a small portion of the dose is involved.

When a 2.5 mg/kg dose was given entirely by the gastrointestinal route, the simulated profile for the vascular compartment lay within the range of jugular venous concentrations observed over most of the first 90 min, but the portal and hepatic venous simulated concentrations were much greater than the observed concentrations for the first 30 min (Figures 6-11 to 6-13). When the dose was reduced to 0.7 mg/kg, the portal and hepatic vein concentration profiles were reasonably well fit but the observed

jugular venous concentrations were much higher than the simulated concentrations for the entire 90 min (Figure 6-13). After addition of 1.8 mg/kg by the pregastric route to the 0.7 mg/kg gastrointestinal dose, the portal venous and hepatic venous concentrations were reasonably well fit by the model although the simulated concentrations were slightly high from about 15 to 45 min. The simulated concentrations in the vascular compartment were slightly above the observed range of concentrations throughout most of the 90 min period. When the pregastric dose was reduced from 1.8 to 1.4 mg/kg to account for the observed incomplete absorption of hydralazine in the dog, the fit to the observed data was improved in all three locations. The best overall fit to the observed data was given by the last condition.

In summary, the simulations show that if the entire dose was absorbed into the portal system, much higher hydralazine concentrations would have been expected than were observed, and if only the amount of drug measured in the portal system was absorbed, systemic concentrations would have been much lower than those observed. Therefore, drug must be entering the systemic circulation through some route not drained by the portal circulation, i.e. the mouth and esophagus. The simulations indicate that pregastric absorption of a large proportion of the administered dose is consistent with the observed data. This hypothesis awaits confirmation in future studies.

If it is found that pregastric absorption of hydralazine can indeed occur after administration of a liquid formulation, then the practice of giving liquid formulations instead of the clinically used dosage forms to improve the reproducibility of oral pharmacokinetic data will be brought into question. Hydralazine is not the only drug which could undergo rapid pregastric absorption and presently employed sampling methods cannot distinguish between absorption routes. Isoproterenol has recently been found to be rapidly absorbed across the buccal membrane (8). The possibility of

pregastric absorption could have an advantage in that new dosage form designs could utilize this property to bypass first pass hepatic metabolism with its inherent bioavailability and toxicological problems.

More minor contributions to the observed discrepancy could occur for other reasons. One source of error may come from the method used to calculate the fraction absorbed. Interpolation by the Lagrange method (3) involves curve smoothing and sharp peaks in the influx *vs.* time curve could be excluded. As well, with such rapid absorption, even 2.5 min sampling intervals may not provide high enough resolution of the curve and again, sharp peaks could be missed. Another way in which a portion of the hydralazine dose absorbed could be missed is that time 0 was set when the dose administration was complete. It took about one minute to administer the drug to a subject, during which some absorption would undoubtedly take place. For a drug which is so rapidly absorbed, the time that was unaccounted for could lead to underestimation of the absorbed amount. In 5 of the 8 dogs, used in the study, initial portal venous concentrations in the fasted condition were greater than 1000 ng/ml. While the importance of this kind of error cannot be accurately evaluated without more data, it must be recognized that the average $t_{max}$ in the portal vein was 6.9 min, so high concentrations would not be unexpected at 2.5 min.

### 6.4.3 Clearance

In Chapter 5, total body clearance for hydralazine was estimated to be 68 ± 8 ml/min/kg after a 2.5 mg/kg dose. In this study, the mean hepatic clearance was calculated to be 11 ± 8 ml/min/kg, less than 20% of $Cl_{tb}$ (Table 6-2). If absorption occurred only in the portions of the gastrointestinal tract drained by the portal vein, the total amount of drug extracted by the liver should be significantly less than the amount absorbed. The total amount of drug extracted by the liver was 12 ± 8 mg in the fasted

state and 11 ± 3 mg in the fed state, however, corresponding to about 25% of the dose given, but 99 ± 39% (fasted) and 115 ± 18% (fed) of the amount absorbed through the portal vein (Table 6-1). Therefore, gastrointestinal absorption cannot account for all the drug extracted by the liver. This constitutes further evidence of pregastric absorption.

### 6.4.4 Portal:Hepatic Venous Lag Time

As already discussed, the 2.5 min samples show that hydralazine may be absorbed from the oral cavity and that there appears to be a lag time between the portal and hepatic venous appearance of drug. The lag time not only interferes with the calculation of pharmacokinetic parameters, but it also indicates the existence of an equilibration process in the liver. While hepatic equilibration is a process that warrants further investigation, studies conducted on isolated livers would yield more useful information.

Another possible consequence arising from an extended hydralazine transit time through the liver is that more time is allowed for intravascular hydrazone formation. If this reaction were to proceed at a higher rate in the fed than in the fasted animal, the lag time alone could account for a reduction in the amount of parent drug exiting the liver, and would reduce $C_{max}$ in the hepatic vein. Counteracting this effect would be the increase in hepatic blood flow associated with ingestion of food. With increased flow rates, the residence time of drug in the liver would be decreased, leading to a reduction in the amount of drug metabolized in the liver.

### 6.4.5 Systemic Parameters

The parameters calculated from jugular venous hydralazine profiles and the differences between nutritional states were generally consistent with the data presented in Chapter 5 and support the conclusions drawn from those data. (See Table 6-2). The reduction in AUC after food was not as great in the present study as in the previous

one, although the variability was high. In this study, $Cl_{oral}$ was significantly increased in the fed state, likely because of the increased number of subjects used.

### 6.4.6 Mechanisms Implicated in the Food Effect

In this experiment, any effect of food on hepatic arterial blood flow was apparently masked by the pharmacological effect of hydralazine, because the mean arterial flow profiles in both conditions were virtually superimposable (Figure 6-5). The main effect of food on hepatic blood flow, over and above that of hydralazine, appears to be the large (about 40%), sustained increase in portal flow. This would be expected to result in decreased hepatic extraction of hydralazine but no such effect was evident. The concomitant increase in $Cl_{int}$ may have compensated for increased flow in the fed state. The increase in $Cl_{int}$ with food, as calculated according to both models, may result from reduced plasma protein binding or increased metabolic capacity in the liver. Hydralazine is loosely but extensively (70% in dog, 87-90% in human, 9 - 10) bound to plasma proteins, and could be displaced by nutrient molecules. This possibility would be very difficult to test in practice because of the extreme lability of hydralazine in plasma. The $t_{1/2}$ of hydralazine is about 10 min *in vitro* in human plasma and all protein binding methods take enough time that considerable decomposition could take place during the measurement. Changes in metabolic capacity can result from changes to $K_m$ or to $V_{max}$ of the metabolic enzymes. Normally, changes to $K_m$ are the result of competition between substrates. The presence of competing food-related substrates would increase $K_m$ and reduce $Cl_{int}$, rather than increasing it as was observed in this study. Changes to $V_{max}$ imply alterations to the capacity of the enzyme system. It has been speculated that this may occur through nutrient-mediated regulation of enzyme activity, such as occurs with the glycolytic enzymes, through changes in the availability of co-substrates such as NADPH or $O_2$ for drug oxidation, or through

changes in substrate delivery rates by alterations in membrane permeability, changes in blood flow patterns through the liver, or active or facilitated transport mechanisms (11-13).

Together, the increases in Q and $Cl_{int}$ in the fed state led to a significant increase (31-61%) in mean hepatic clearance of hydralazine. The increase in clearance appeared to be most pronounced early in the experiments, during peak absorption periods, and could contribute to the decrease in hydralazine AUC seen with food administration. The clearance values observed in these experiments, however, even at their peaks, around 23 ml/min/kg, were only one third of the total body clearance recorded in our earlier work. After 2.5 mg/kg intravenous doses of hydralazine, the mean $Cl_{tb}$ was $68 \pm 4$ ml/min/kg. Thus, hepatic clearance may be responsible for a maximum of one third of the elimination of hydralazine. Furthermore, this proportion likely remained similar in the fed state. Neither the mean amount of drug extracted by the liver nor the amount of drug absorbed changed significantly between the fasted and fed states, even though hepatic clearance increased. If there was no concomitant increase in the amount extracted by the liver, then total body clearance must have increased by roughly the same degree. This may be supported by data from the present study in that mean $Cl_{oral}$ was about 5 times higher than mean $Cl_{int}$. Both increased by similar degrees ($Cl_{int}$ increased by 60%, $Cl_{oral}$ by 80%) after food. While the latter reflects values associated only with the liver, the AUC of orally administered hydralazine, from which the $Cl_{oral}$ term was derived, can be affected by absorption, hepatic metabolism and extrahepatic elimination. Nevertheless, it would appear that a large proportion of the total body clearance occurs outside the liver, in the systemic compartment. Ludden *et al.* (14) recorded a large increase in blood levels of the intravascularly formed metabolite, hydralazine pyruvic acid hydrazone associated with a meal in humans. They attributed the reduction in hydralazine AUC in humans after a meal to increased formation of this

metabolite secondary to elevated blood levels of the co-substrate, pyruvic acid associated with food ingestion. Plasma pyruvic acid in the fasted human, however, is present in at least a five-fold molar excess (50 mM/*l*) over peak portal concentrations (~10 mM/*l*) and a ten-fold excess over maximum systemic blood concentrations of hydralazine; and pyruvic acid levels increase by 4 times to about 200 mM/*l* after a meal in humans (15). While this may contribute to the increased production of the hydrazone, it is hydralazine which is likely to be the limiting substrate. It is reported to be highly bound to plasma proteins, so the excess of pyruvate over free hydralazine could be even larger than was calculated. A reduction of hydralazine protein binding with food would increase free hydralazine levels and thus the availability of the drug to react with pyruvate. A reduction in plasma protein binding of hydralazine could account for both the increase in hepatic intrinsic clearance and pyruvic acid hydrazone formation, especially when they are both increased to similar extents after food. As previously mentioned, this mechanism would be very difficult to measure. While an increase in systemic clearance of hydralazine is likely to be the major mechanism of the food effect on hydralazine kinetics, the increase in hepatic clearance also contributes significantly to the total body clearance. It is apparent that the effect of food on hydralazine kinetics is extremely complex and the peripheral blood concentration *vs.* time profile is the sum of several coincident effects on almost every pharmacokinetic parameter.

### 6.4.7 Animal Model Evaluation

This animal preparation allows the direct measurement of hepatic events which can occur only in the conscious animal. The advantages over anesthetized animal preparations and isolated perfused liver preparations are that all the neurological, endocrine and physiological control mechanisms remain intact, but physiological

modelling parameters can nevertheless be measured and the contributions of different mechanisms evaluated. As well, the subject is physically able to ingest and digest food so its effect can be directly measured. The animals are essentially healthy and normal. The similarities between peripheral blood data from unaltered dogs in the pilot study and the surgically prepared animals support the validity of the assumption that the surgery does not significantly alter the pharmacokinetics of hydralazine.

Despite the advantages of this method, some problems must be recognized. The preparation is very costly. If the necessary pharmacokinetic information can be gathered in another way, it is probably less expensive. This statement must be tempered by the assertion that many different parameters can be evaluated simultaneously, which adds to the cost effectiveness of the preparation. The surgical technique is demanding, and success is not uniform. If only one catheter becomes blocked or one flow probe malfunctions, the entire preparation is usually rendered useless. The success rate improves vastly with experience.

Because the animal subjects are conscious, the data tends to be more variable than with other preparations. Flows change when the subjects change positions or when external stimuli are introduced. The subjects can become quite restless, especially during fasted experiments. Statistical power and resolution is therefore somewhat lower than for other preparations and ways must be found to increase the number of data points when comparing samples. This can be done by increasing the number of subjects tested and by pooling data from several time points, where applicable.

Rapidly changing phenomena are not accurately depicted by this model, because of high variability, time lags between sampling locations and because data cannot be pooled to increase statistical power. Therefore, events such as transient saturation of metabolism could not be detected. Experiments conducted under steady-state conditions with constant infusions of drug or nutrients, or the use of drugs which are more slowly

absorbed and distributed could increase the power of the conscious animal preparation. Because the *in vivo* system is a recirculating one, metabolite-parent drug interactions can occur, complicating the interpretation of results.

The simultaneous removal of blood from four sites can result in significant blood loss to the subjects. Therefore, the sample size and number of samples taken must be limited.

In summary, pregastric absorption of a large proportion of a hydralazine dose may occur when a liquid formulation is administered. Food caused changes to $Q_h$ and $Cl_{int}$ which resulted in increased hepatic clearance of hydralazine. The major mechanism of the food effect may be an increase in the systemic clearance of hydralazine.

## 6.5 Tables

Table 6-1. Body weight, hydralazine dose, amount absorbed into the PV, fraction of total dose absorbed, amount extracted by the liver, and amount extracted / amount absorbed into the PV, in fasted and fed states, in 8 dogs (K60, K90 had no HV catheter).

| Fasted | | | | | | |
|---|---|---|---|---|---|---|
| Dog # | Body Weight (kg) | Total Dose HP base (mg) | Amount Absorbed (mg) | Fraction Absorbed (of total dose) | Amount Extracted (mg) | Amount Extracted /Amount Absorbed (%) |
| J598 | 18.0 | 36.7 | 13.8 | 0.38 | 13.33 | 97 |
| K170 | 23.5 | 47.9 | 5.7 | 0.12 | 2.36 | 41 |
| K154 | 22.0 | 44.8 | 5.0 | 0.11 | 7.31 | 146 |
| K59 | 23.5 | 47.9 | 8.6 | 0.18 | 6.26 | 73 |
| K189 | 21.5 | 43.8 | 14.3 | 0.33 | 19.62 | 137 |
| K178 | 22.5 | 45.8 | 22.7 | 0.50 | 22.97 | 101 |
| K60 | 23.0 | 46.8 | 21.0 | 0.45 | | |
| K90 | 21.5 | 43.8 | 7.8 | 0.18 | | |
| Mean | 21.9 | 44.7 | 12.4 | 0.28 | 11.98 | 99 |
| SD | 1.8 | 3.6 | 6.8 | 0.15 | 8.10 | 39 |
| Fed | | | | | | |
| J598 | 18.0 | 36.7 | 9.9 | 0.27 | 12.51 | 126 |
| K170 | 23.5 | 47.9 | 6.2 | 0.13 | 7.06 | 114 |
| K154 | 22.0 | 44.8 | 7.5 | 0.17 | 7.45 | 99 |
| K59 | 23.5 | 47.9 | 13.3 | 0.28 | 14.81 | 111 |
| K189 | 21.5 | 43.8 | 8.9 | 0.20 | 12.82 | 144 |
| K178 | 22.5 | 45.8 | 13.7 | 0.30 | 12.93 | 94 |
| K60 | 23.0 | 46.8 | 5.4 | 0.12 | | |
| K90 | 21.5 | 43.8 | 7.1 | 0.16 | | |
| Mean | 21.9 | 44.7 | 9.0 | 0.20 | 11.26 | 115 |
| SD | 1.8 | 3.6 | 3.1 | 0.07 | 3.21 | 18 |

Table 6-2. Hepatic extraction ratio, mean hepatic clearance, and hepatic intrinsic clearance, calculated according to the WS and PT models in the fasted and fed states in 6 dogs.

| Fasted | | | | |
|---|---|---|---|---|
| Dog # | Hepatic Extraction Ratio | Mean Hepatic Clearance (ml/min/kg) | $Cl_{int}$, WS (ml/min/kg) | $Cl_{int}$, PT (ml/min/kg) |
| J598 | 0.49 | 14 | 30 | 20 |
| K170 | 0.18 | 6 | 11 | 8 |
| K154 | 0.40 | 9 | 16 | 12 |
| K59 | 0.18 | 7 | 11 | 9 |
| K189 | 0.69 | 16 | 42 | 24 |
| K178 | 0.66 | 14 | 33 | 20 |
| Mean | 0.43 | 11 | 24 | 15 |
| SD | 0.22 | 4 | 13 | 7 |
| Fed | | | | |
| J598 | 0.80 | 21 | 47 | 28 |
| K170 | 0.51 | 9 | 18 | 12 |
| K154 | 0.45 | 14 | 22 | 17 |
| K59 | 0.55 | 10 | 19 | 13 |
| K189 | 0.62 | 26 | 66 | 38 |
| K178 | 0.60 | 22 | 59 | 34 |
| Mean | 0.59 | 17 | 38 | 24 |
| SD | 0.12 | 7 | 22 | 11 |

Table 6-3. Peripheral blood (JV) pharmacokinetic parameters measured in fasted and fed states in 8 dogs.

| Dog # | AUC (ng-min/ml) | Relative F | $Cl_{oral}$ (ml/min/kg) | β (1/min) | $t_{1/2}$ (min) | MRT (min) |
|---|---|---|---|---|---|---|
| Fasted | | | | | | |
| J598 | 21522 | | 95 | 0.0051 | 135 | 71 |
| K170 | 17989 | | 113 | 0.0056 | 123 | 102 |
| K154 | 20058 | | 102 | 0.0037 | 188 | 127 |
| K59 | 29291 | | 70 | 0.0038 | 182 | 101 |
| K189 | 18584 | | 110 | 0.0048 | 141 | 78 |
| K178 | 15513 | | 131 | 0.0034 | 205 | 145 |
| K60 | 28138 | | 72 | 0.0051 | 135 | 79 |
| K90 | 23613 | | 86 | 0.0068 | 101 | 150 |
| Mean | 21839 | | 97 | 0.0048 | 151 | 107 |
| SD | 4885 | | 21 | 0.0011 | 36 | 31 |
| Fed | | | | | | |
| J598 | 9655 | 0.45 | 211 | 0.0130 | 50 | 131 |
| K170 | 14397 | 0.80 | 141 | 0.0064 | 108 | 95 |
| K154 | 10260 | 0.51 | 199 | 0.0048 | 144 | 138 |
| K59 | 19400 | 0.66 | 105 | 0.0048 | 145 | 84 |
| K189 | 9678 | 0.52 | 210 | 0.0058 | 119 | 96 |
| K178 | 8589 | 0.55 | 237 | 0.0069 | 100 | 129 |
| K60 | 11932 | 0.42 | 171 | 0.0051 | 136 | 100 |
| K90 | 14095 | 0.60 | 145 | 0.0067 | 104 | 170 |
| Mean | 12251 | 0.56 | 177 | 0.0067 | 113 | 118 |
| SD | 3585 | 0.12 | 45 | 0.0027 | 31 | 29 |

Table 6-4. $C_{max}$ and $t_{max}$ from the portal vein (PV), hepatic vein (HV), carotid artery (CA) and jugular vein (JV) in fasted and fed states in 8 dogs.

| Fasted | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dog # | $C_{max}$ (PV) (ng/ml) | $t_{max}$ (PV) (min) | $C_{max}$ (HV) (ng/ml) | $t_{max}$ (HV) (min) | $C_{max}$ (CA) (ng/ml) | $t_{max}$ (CA) (min) | $C_{max}$ (JV) (ng/ml) | $t_{max}$ (JV) (min) |
| J598 | 2559 | 10.0 | 893 | 10.0 | 529 | 20.0 | 784 | 15.0 |
| K170 | 2771 | 5.0 | 1455 | 7.5 | 720 | 7.5 | 503 | 10.0 |
| K154 | 882 | 15.0 | 376 | 10.0 | 388 | 10.0 | 260 | 20.0 |
| K59 | 3370 | 5.0 | 2444 | 5.0 | 1764 | 5.0 | 955 | 5.0 |
| K189 | 3331 | 8.0 | 626 | 15.0 | 824 | 11.0 | 525 | 15.0 |
| K178 | 4215 | 2.5 | 786 | 5.0 | 673 | 7.5 | 375 | 15.0 |
| K60 | 4827 | 7.5 | | | 2378 | 10.0 | 744 | 15.0 |
| K90 | 1491 | 2.5 | | | 411 | 5.0 | 341 | 25.0 |
| Mean | 2931 | 6.9 | 1097 | 8.8 | 961 | 9.5 | 561 | 15.0 |
| SD | 1311 | 4.2 | 751 | 3.8 | 720 | 4.8 | 244 | 6.0 |
| Fed | | | | | | | | |
| J598 | 2701 | 5.0 | 171 | 10.0 | 260 | 10.0 | 336 | 10.0 |
| K170 | 1560 | 5.0 | 532 | 7.5 | 388 | 7.5 | 247 | 15.0 |
| K154 | 558 | 15.0 | 214 | 15.0 | 189 | 15.0 | 123 | 15.0 |
| K59 | 2813 | 7.5 | 998 | 10.0 | 606 | 10.0 | 590 | 7.5 |
| K189 | 853 | 5.0 | 235 | 15.0 | 368 | 10.0 | 229 | 15.0 |
| K178 | 799 | 10.0 | 206 | 15.0 | 130 | 20.0 | 106 | 20.0 |
| K60 | 1577 | 7.5 | | | 609 | 10.0 | 375 | 15.0 |
| K90 | 639 | 15.0 | | | 192 | 20.0 | 143 | 25.0 |
| Mean | 1438 | 8.8 | 393 | 12.1 | 343 | 12.8 | 269 | 15.3 |
| SD | 901 | 4.2 | 325 | 3.3 | 186 | 4.9 | 162 | 5.4 |

## 6.6 Figures

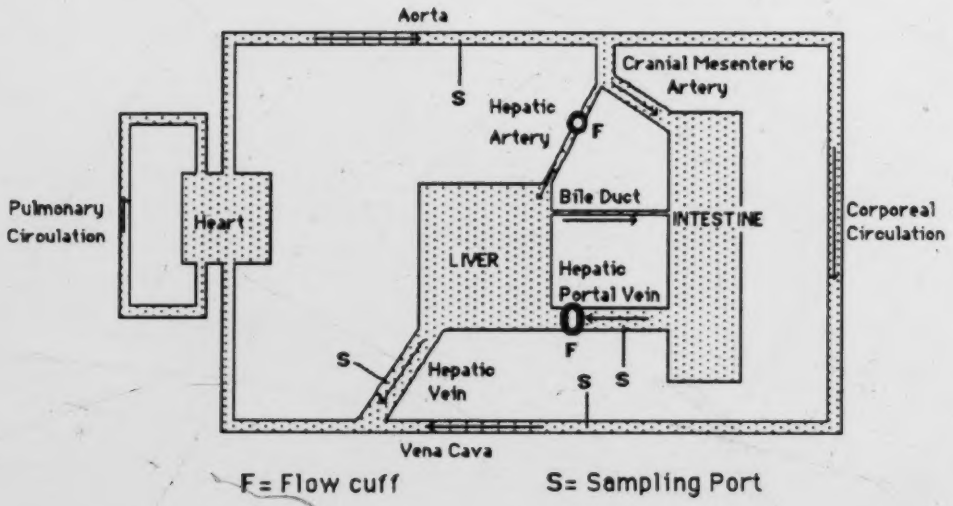


Figure 6-1. Schematic representation of circulation of the body showing locations of sampling catheters and flow probes.

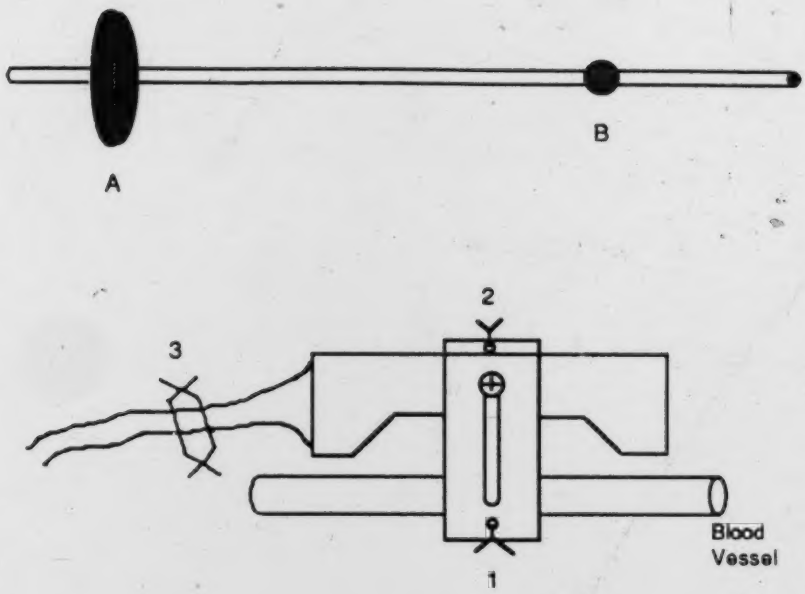


Figure 6-2. Implantable catheter showing subcutaneous dacron velour flange (A) and anchor point (B), flow probe showing standard suture placements for anchoring to surrounding tissues (1, 2, 3). A vessel is seen to pass through the probe.

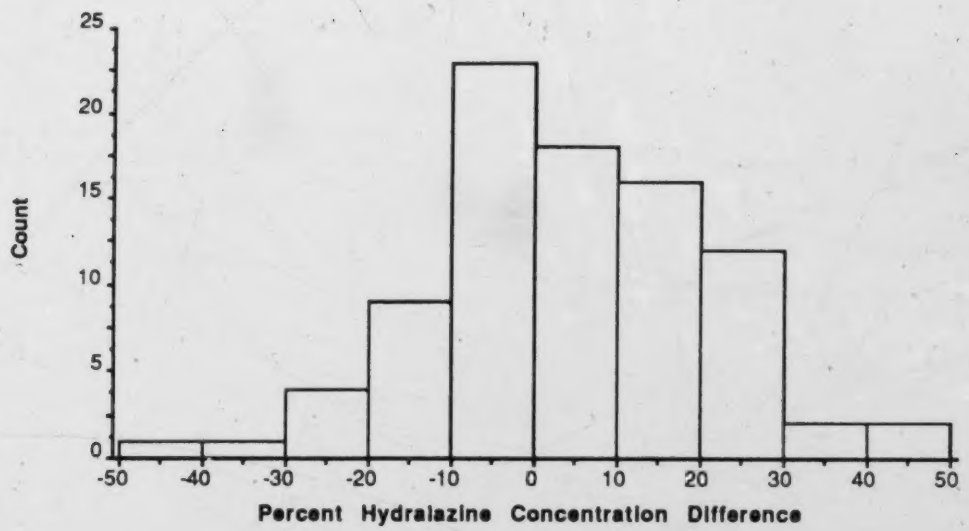


Figure 6-3. Percent differences in hydralazine concentration measured simultaneously in 2 catheters placed in different locations in the portal vein.

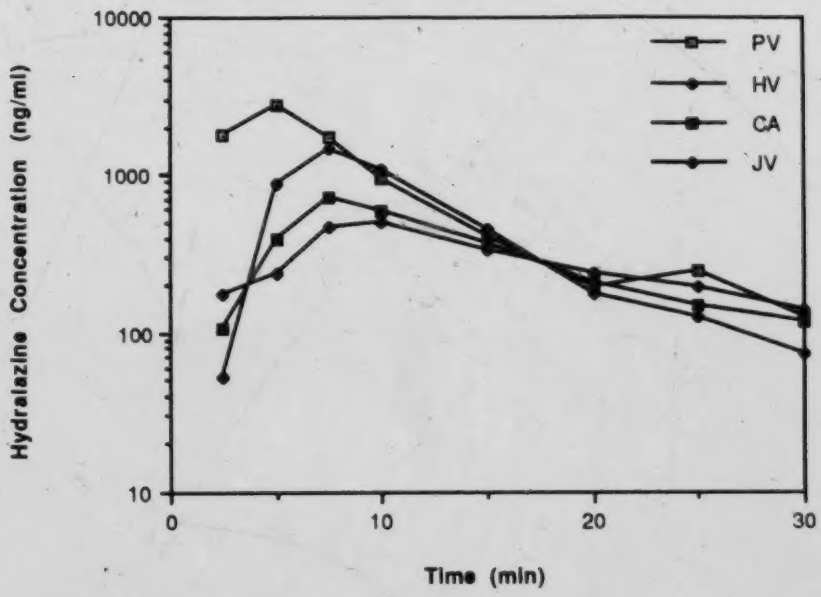


Figure 6-4. Representative plot of concentration *vs*. time in the four vessels, from subject K170 (fasted condition).

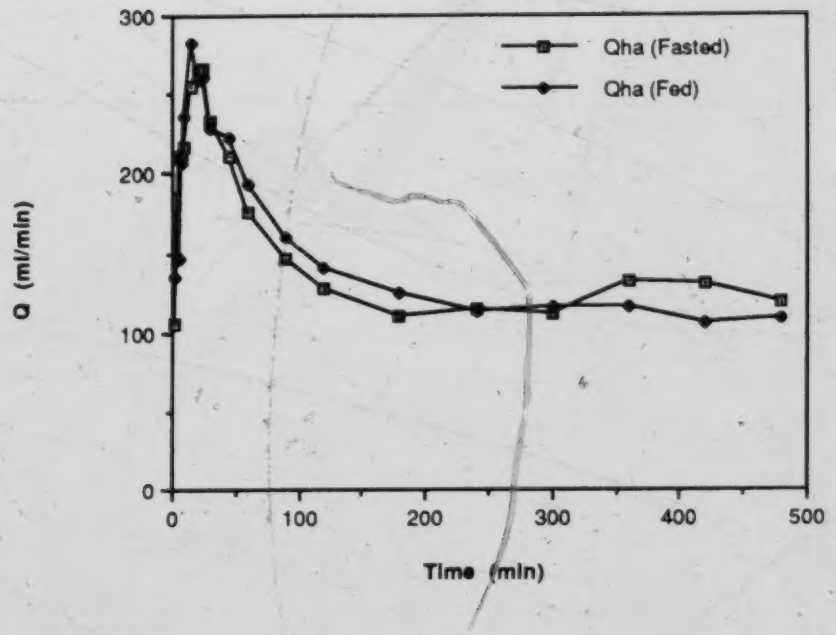


Figure 6-5. Mean hepatic arterial blood flow *vs*. time, Fasted *vs*. Fed Conditions.

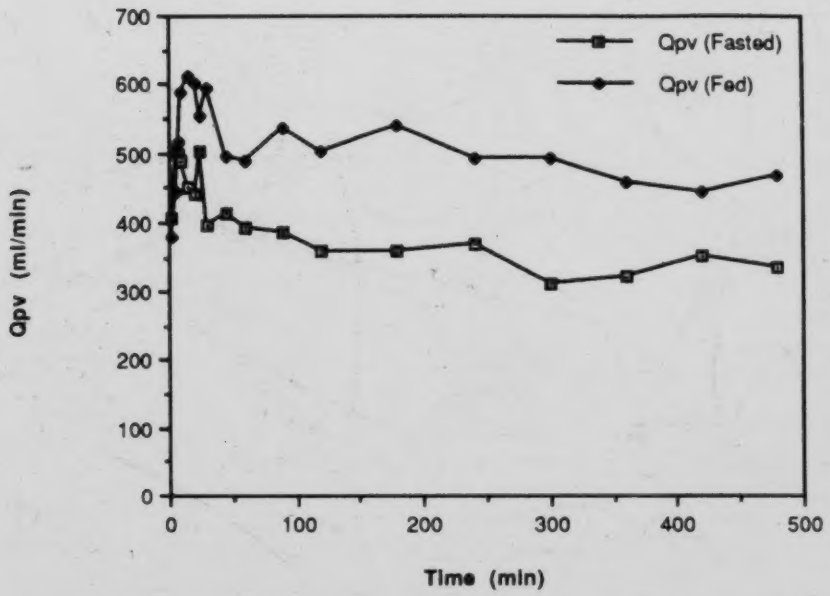


Figure 6-6. Mean portal venous blood flow *vs*. time, Fasted *vs*. Fed conditions.

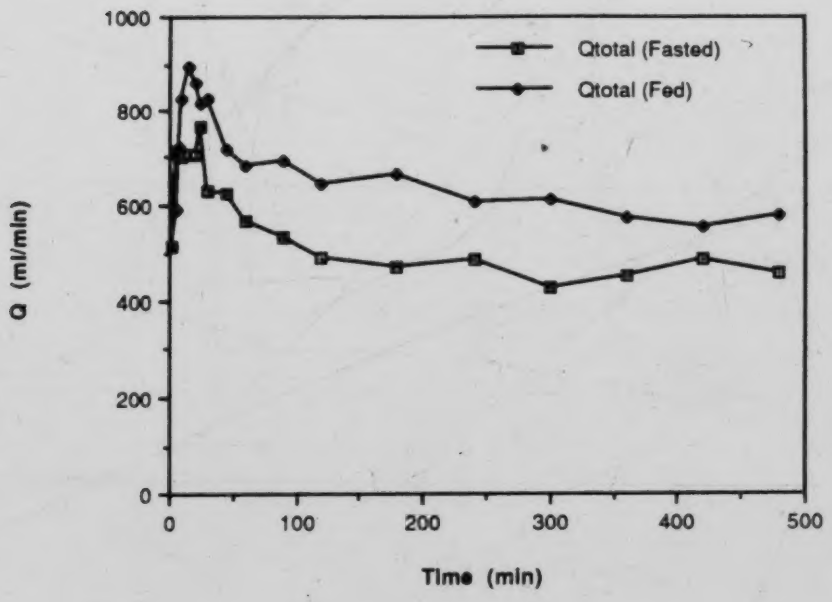


Figure 6-7. Mean total hepatic blood flow *vs*. time, Fasted *vs*. Fed conditions.

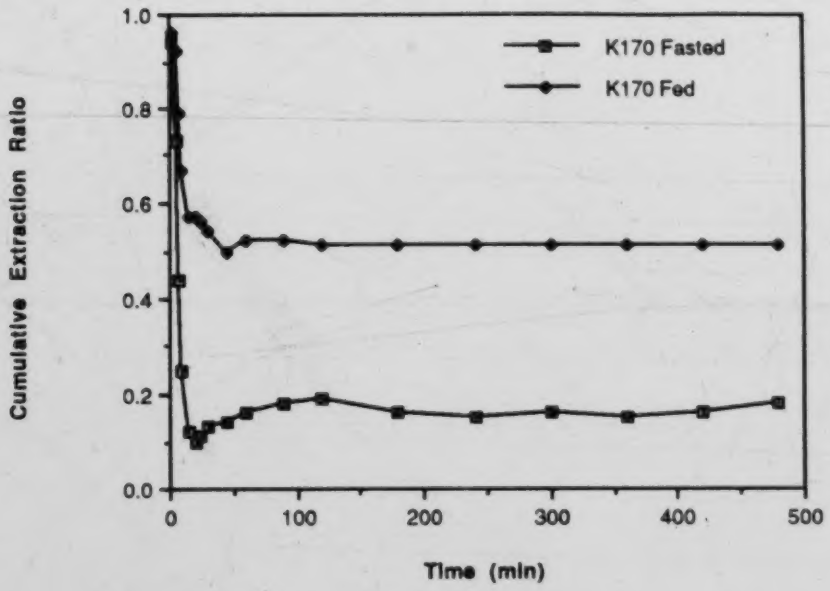


Figure 6-8. Cumulative extraction ratio *vs*. time for subject K170, Fasted *vs*. Fed.

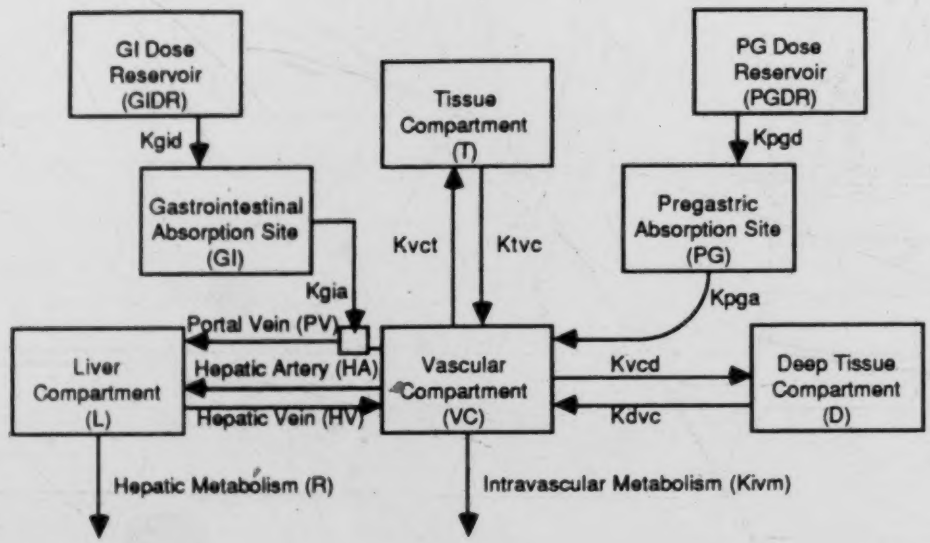


Figure 6-9. Schematic diagram of simulation model layout. See text (Appendix 1) for definition of terms.

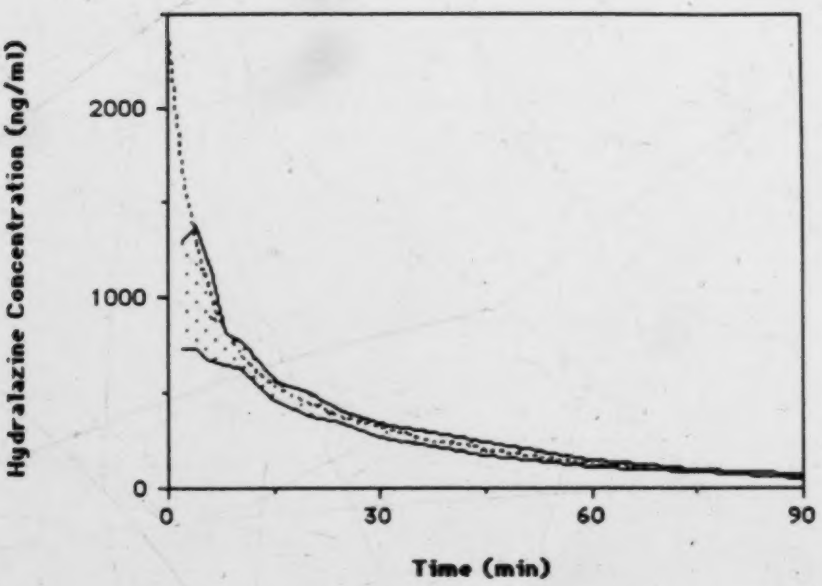


Figure 6-10. Simulated concentration *vs.* time curve in the peripheral blood (Vascular Compartment) after a 2.5 mg/kg iv dose of hydralazine, compared with the area covered by observed data.

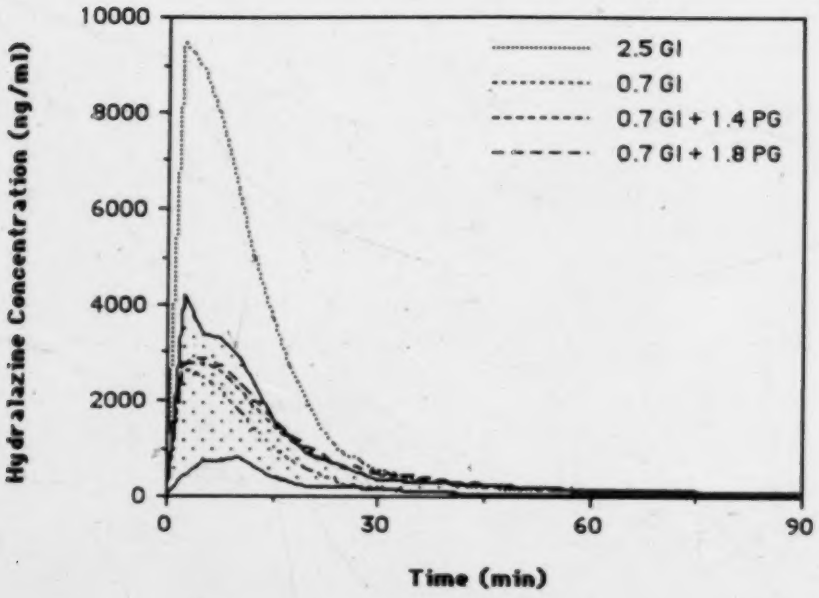


Figure 6-11. Simulated concentration *vs.* time curves in the portal vein after doses of hydralazine introduced into the gastrointestinal compartment (GI) or GI plus pregastric compartment (PG), compared with the area covered by observed data after 2.5 mg/kg doses in 6 fasted dogs. The doses (mg/kg) and routes used for the simulations are given in the legend.

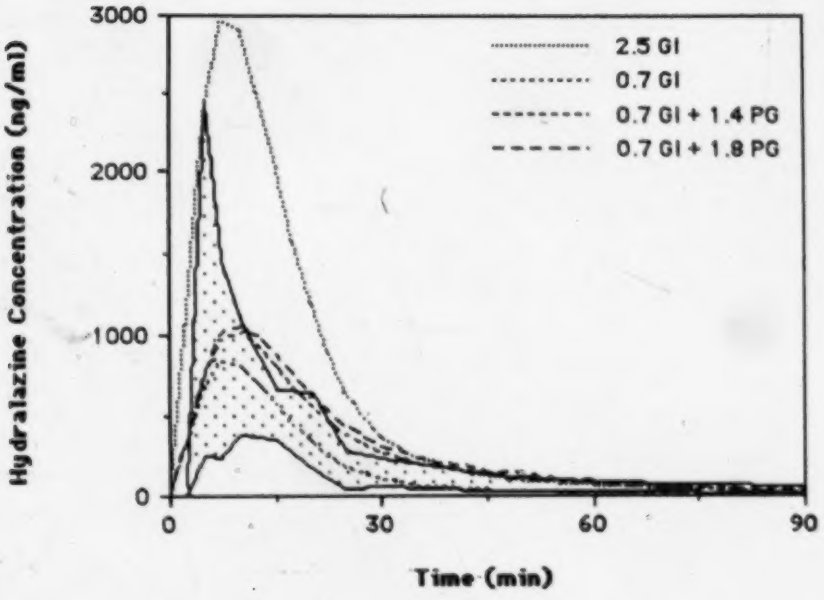


Figure 6-12. Simulated concentration *vs.* time curves in the hepatic vein after doses of hydralazine introduced into the gastrointestinal compartment (GI) or GI plus pregastric compartment (PG), compared with the area covered by observed data after 2.5 mg/kg doses in 6 fasted dogs. The doses (mg/kg) and routes used for the simulations are given in the legend.

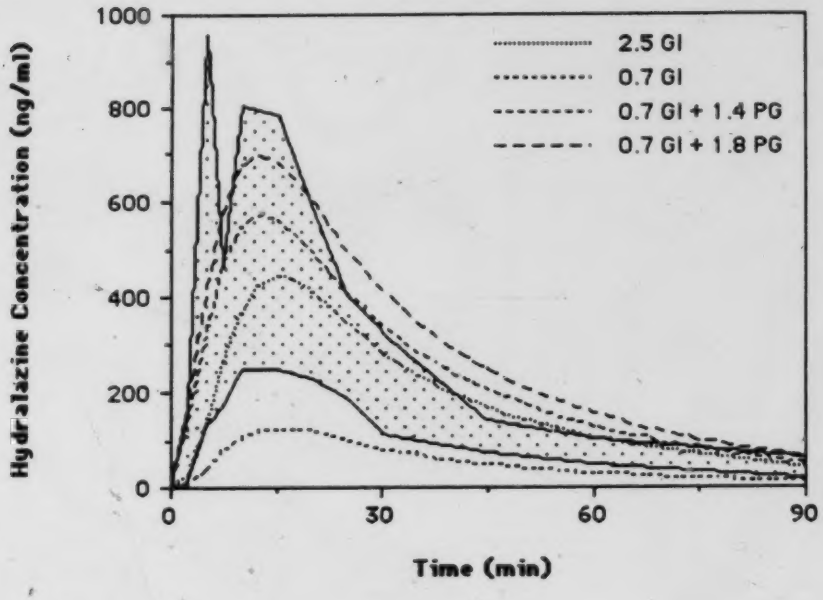


Figure 6-13. Simulated concentration *vs.* time curves in the jugular vein (Vascular Compartment) after doses of hydralazine introduced into the gastrointestinal compartment (GI) or GI plus pregastric compartment (PG), compared with the area covered by observed data after 2.5 mg/kg doses in 6 fasted dogs. The doses (mg/kg) and routes used for the simulations are given in the legend.

## 6.7 References


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# 7. Interactions Between Hydralazine and Oral Nutrients in Humans

## 7.1 Introduction

There has been controversy surrounding the effect of food on the pharmacokinetics of hydralazine, with reports of increased (1, 2), no change (3) and decreased (4-7) bioavailability. It is difficult to compare the information from the various studies because of a number of confounding factors, including differences in meal composition, timing of drug administration in relation to meal intake, acetylator status of the subjects, type of drug formulation, and specificity of the drug assay (1-9).

We have recently developed a sensitive, specific HPLC assay (Chapter 2) for hydralazine and data from the dog model indicate that bioavailability is decreased when the drug is co-administered with food (Chapters 5, 6).

Patients taking drugs which interact with food are occasionally co-administered enteral nutrients. Whether these purified nutrient formulations interact with drugs in the same way as normal food is unknown, as is the effect of the rate and mode of delivering them. Since patients receiving enteral nutrients are already compromised in some way, it is doubly important that concomitantly administered drugs are dosed so that concentrations fall within the "therapeutic window". For this to be accomplished, the effect of these nutrients on the various pharmacokinetic parameters must be known.

This study is aimed toward clarifying the issue of the food effect on hydralazine kinetics by testing if administration of the drug during ingestion of a meal will decrease its bioavailability. In addition, whether "bolus" ingestion of nutrients compared to delivery of the same nutrient as a continuous infusion via nasogastric tube would result in a lower bioavailability of hydralazine was investigated.

## 7.2 Methods

The study was approved by the local ethics committee and was performed at the Clinical Investigation Unit, University of Alberta Hospital. Four healthy males and four healthy females, aged 27.1 ± 5.7 yr, weighing 71.6 ± 11.7 kg, with height 174 ± 7 cm, volunteered as test subjects. None were smokers. Standard liver function tests and routine blood work were normal in all. Each subject took hydralazine on four separate occasions: during fasting conditions, together with a standardized breakfast (containing 470 ml liquid volume, plus 17.4 - 17.6 g protein, 17.4 g fat, and 68.2 g carbohydrate to give 500 kcal, consumed over 20 min), together with a 470 ml bolus of complete liquid nutrition (500 kcal, 17.5 g protein, 17.5 g fat, 68.2 g carbohydrate, Ensure, Ross Laboratories, Montreal, Canada, consumed over 20 min), or during a 6 h infusion of 470 ml of complete liquid nutrition by nasogastric tube. The macronutrient content of the diets is similar to those in previous studies (1,4). Subjects were randomly assigned to two 4 x 4 Latin Squares of subject x treatment.

At least 7 days elapsed between tests. Before each test, the subjects abstained from food and liquid for at least 10 h. On the mornings of the tests, each subject remained at bed rest for 1 h prior to dosing and for 2 h after dosing. Heart rate and blood pressure were monitored at 10 min intervals for 1 h prior to commencement of the study and for 2 h after drug ingestion, then every 30 min until the end of the study. Other than the nutrients provided, the subjects were allowed nothing *per os* until 2 h post-dosing, when they were allowed water only for the duration of the experiment.

Three ml blood samples were collected into disposable plastic syringes whose hubs contained 0.1 M EDTA as an anticoagulant and immediately transferred into borosilicate glass screw-capped tubes with PTFE-lined caps for hydralazine analysis. Blood hydralazine concentration was measured according to our newly-developed,

specific method which involves immediate derivatization with p-nitrobenzaldehyde (Chapter 2).

After collection of blank blood samples and 10 min after commencement of nutrient intake, 50 mg of hydralazine HCl solution (Apresoline, Ciba Geigy, Lot 5700) was administered orally, followed by 100 ml of water. Blood samples were collected at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, and 360 min after drug ingestion. Extraction residues were stored at -20° C until analysis.

Subjects were acetylator phenotyped using the molar ratio of 5-acetylamino-6-formylamino-3-methyluracil (AFMU, supplied by Dr. B. Tang, Department of Pharmacology, University of Toronto, Toronto, ON) to 1-methylxanthine (1MX, Sigma Chemical Co., St. Louis, MO) in urine collected about 4 h after consumption of a caffeinated beverage (10, 11). The molar ratio of AFMU:1MX was calculated without an internal standard (10) and the antimode of about 0.5 was used as the division between slow and fast acetylators. The elution times of caffeine metabolite standards matched published values very closely (10). The metabolites included AFMU, 3-methyluric acid, 7-methyluric acid, 3-methylxanthine, 1-methylxanthine, 1, 3-dimethyluric acid, 3, 7-dimethylxanthine, 1, 7-dimethylxanthine, 1, 3-dimethylxanthine, and 1, 3, 7-trimethyluric acid. Except for the AFMU, all metabolites were from Sigma Chemical Co., St. Louis, MO.

Pharmacokinetic analyses of concentration *vs.* time data were carried out using non-compartmental methods (12), and the data were fit using the computer program, LAGRAN (13). The Latin squares were analyzed by repeated measures analyses of variance with Fisher PLSD tests used for post hoc comparisons of means. $\alpha$ was set at $p = 0.05$.

## 7.3 Results

The pharmacokinetic data are summarized in Table 7-1, and the concentration *vs.* time profiles from the patients are shown in Figures 7-1 to 7-8. Note that, due to the variability in concentrations, the concentration scales differ considerably amongst the profiles. The $AUC_{0\text{-}6\ h}$ was approximately halved and $C_{max}$ was reduced by about 80% when a bolus of nutrients was given, either in the form of a standard breakfast or the enteral nutrition product; both changes were statistically significant ($p < 0.05$). No significant change from the fasted condition occurred when the enteral nutrients were given as an infusion ($p > 0.05$). $T_{max}$ remained unchanged throughout the treatments, but MRT after a bolus of enteral nutrients was approximately doubled compared with the fasted or enteral infusion conditions ($p < 0.05$).

The variability within each treatment group was large and was related to acetylation phenotype. Figure 7-9 shows a scatter plot of $AUC_{0\text{-}6\ h}$ *vs.* AFMU/1MX molar ratio. Two distinct groups are evident, corresponding to the two acetylator phenotypes. The AFMU/1MX molar ratio division between slow and fast acetylators was approximately 0.5. Subjects B, C, D, G and H were classified as slow acetylators and A, E and F were fast acetylators. There was no apparent sex-related variability between groups.

## 7.4 Discussion

Ingestion of a bolus of nutrients very clearly reduced hydralazine availability in this group of subjects. The data are in agreement with the observations of Shepherd *et al.* (4-6) and Ludden *et al.* (7), and follow the pattern observed earlier in the dog (Chapters 5, 6). $C_{max}$ was reduced to a greater extent than AUC, without a significant change in $t_{max}$. This may indicate the substantial contribution of a presystemic component to the food effect. While Ludden *et al.* (7) have stressed the importance of a

food related increase in the systemic metabolism of hydralazine, studies in the dog (Chapter 6) have shown that hepatic clearance of hydralazine also increases with food ingestion, secondary to increases in portal venous blood flow and hepatic intrinsic clearance. These may be important mechanisms in the human as well.

The effect of nutrients infused over 6 h was minimal, indicating that the rate of nutrient delivery is an important factor in activating the mechanisms of the food effect. Nutrient delivery rate could influence drug bioavailability in patients receiving both drug therapy and enteral nutrition. In addition, if the infusion was to be initiated earlier, the results are unknown. More studies in this area are warranted.

The similarity between the effects of the standard breakfast and enteral bolus indicate that the physical form of the nutrients may not play an important role in determining the bioavailability of hydralazine. Efforts should therefore be concentrated on determining the contribution of each nutrient to the food effect. The ability to influence bioavailability with purified nutrients will simplify the formulation of test meals.

MRT was significantly higher after an enteral bolus than in the fasting or enteral infusion conditions ($p < 0.05$), even though $t_{max}$ was not significantly affected. This is partially related to more sustained hydralazine blood levels, suggesting that absorption rates are slower under this condition.

The caffeine metabolite method of determining acetylation phenotypes appeared to correctly separate the eight patients into fast and slow metabolizers of hydralazine. This method may be a reliable indicator of hydralazine acetylation phenotype, however, it will be necessary to test many more subjects before this can be confirmed.

This study demonstrates that, in addition to earlier recommendations that hydralazine be taken at a fixed time interval with meals (2, 4), dosage adjustment should be considered in patients receiving hydralazine and enteral nutrition

concomitantly. The effect of enterally administered nutrients on the pharmacokinetics of other drugs subject to extensive first pass metabolism is at present unknown.

## 7.5 Table

Table 7-1: Pharmacokinetic Parameters for subjects given oral hydralazine under different nutritional conditions Mean (± SD)

| Treatment | $AUC_{0\text{-}6\ h}$ (ng.min/ml) | $C_{max}$ (ng/ml) | $t_{max}$ (min) | MRT (min) |
|---|---|---|---|---|
| Fasted | 2516 (1924) | 88 (80) | 26 (6) | 85 (20) |
| Standard Breakfast | 982 (607) | 16 (12) | 30 (25) | 113 (26) |
| Enteral Infusion | 2627 (1693) | 111 (86) | 20 (7) | 79 (32) |
| Enteral Bolus | 1265 (1159) | 18 (18) | 46 (28) | 155 (81) |

## 7.6 Figures

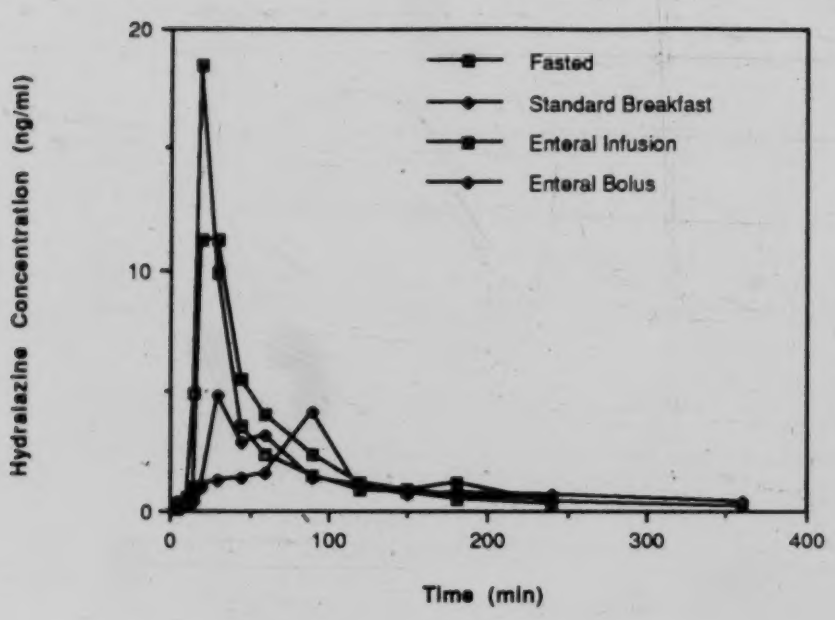


Figure 7-1. Hydralazine concentration *vs.* time profiles for Patient A (fast acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

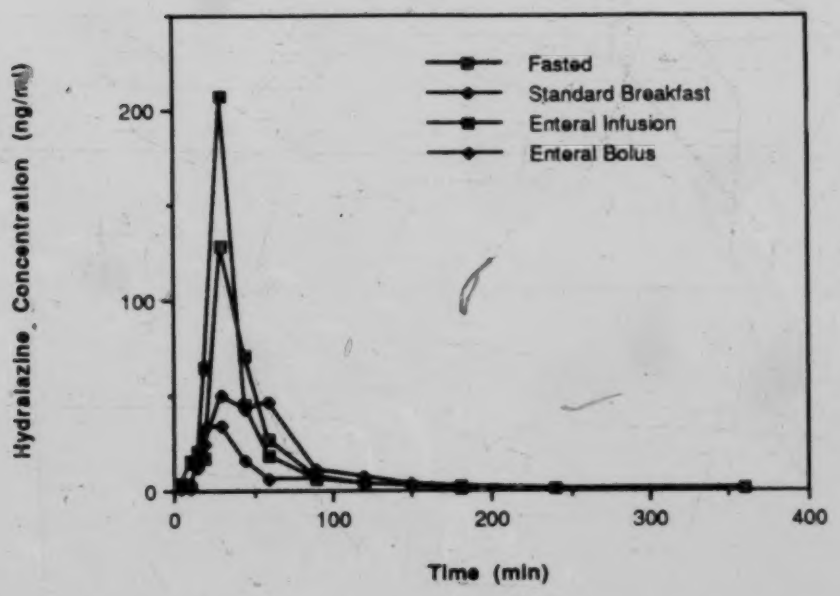


Figure 7-2. Hydralazine concentration *vs.* time profiles for Patient B (slow acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

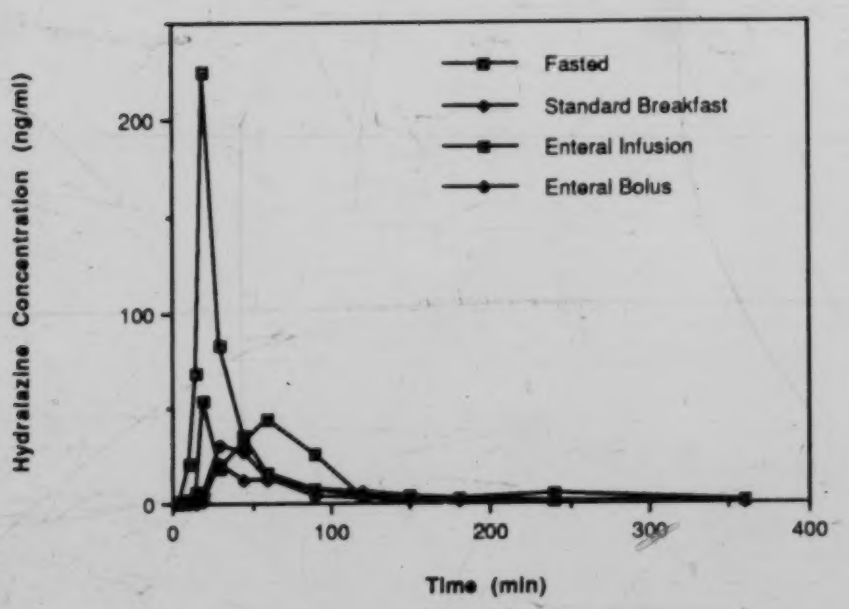


Figure 7-3. Hydralazine concentration *vs.* time profiles for Patient C (slow acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

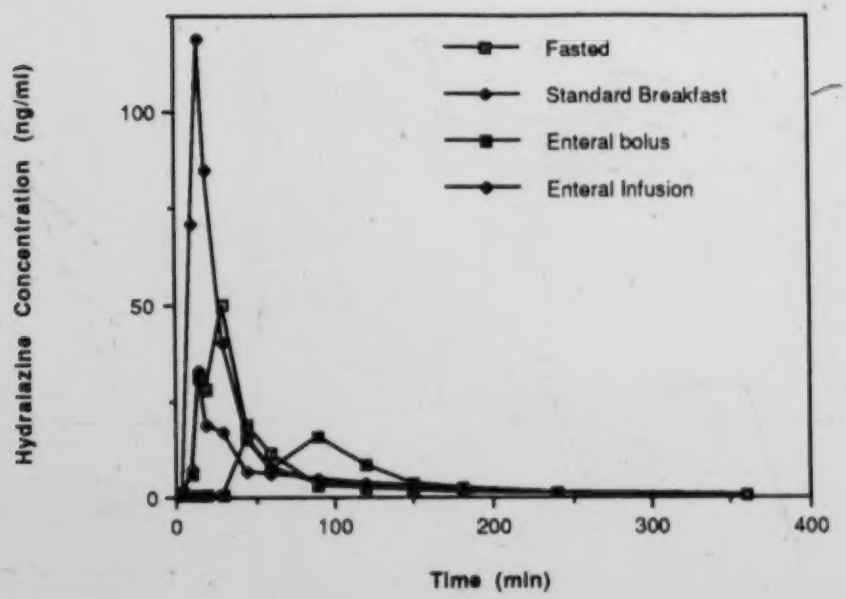


Figure 7-4: Hydralazine concentration *vs.* time profiles for Patient D (slow acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

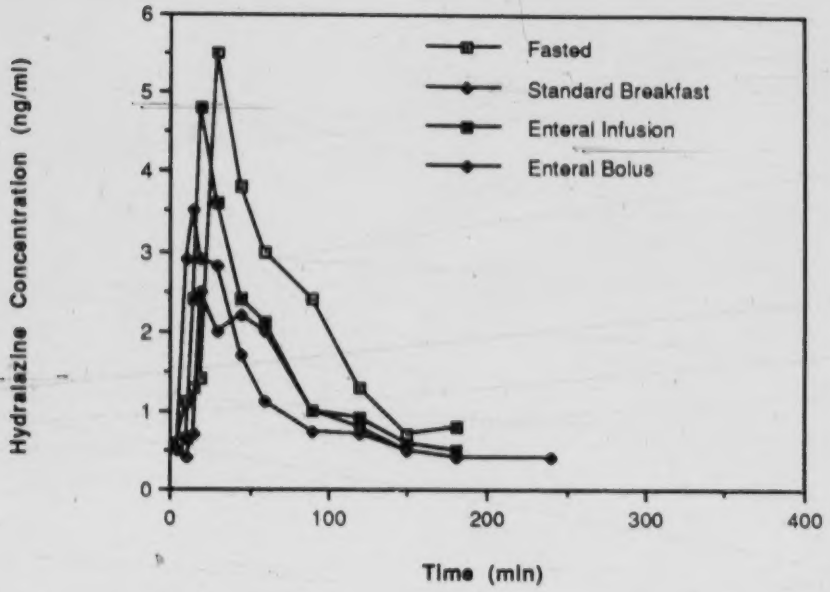


Figure 7-5: Hydralazine concentration *vs.* time profiles for Patient E (fast acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

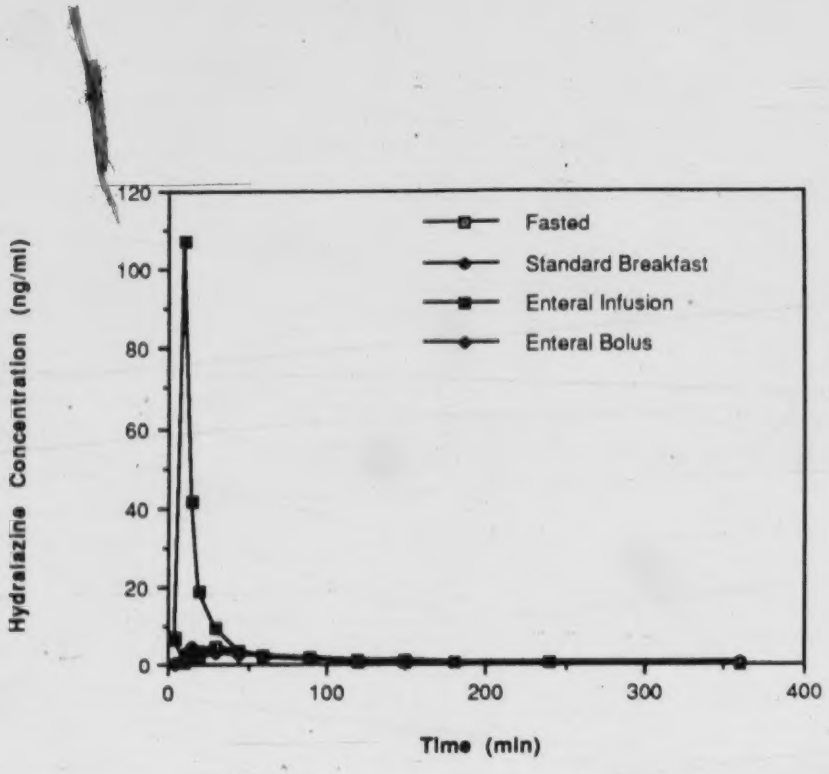


Figure 7-6: Hydralazine concentration *vs.* time profiles for Patient F (fast acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

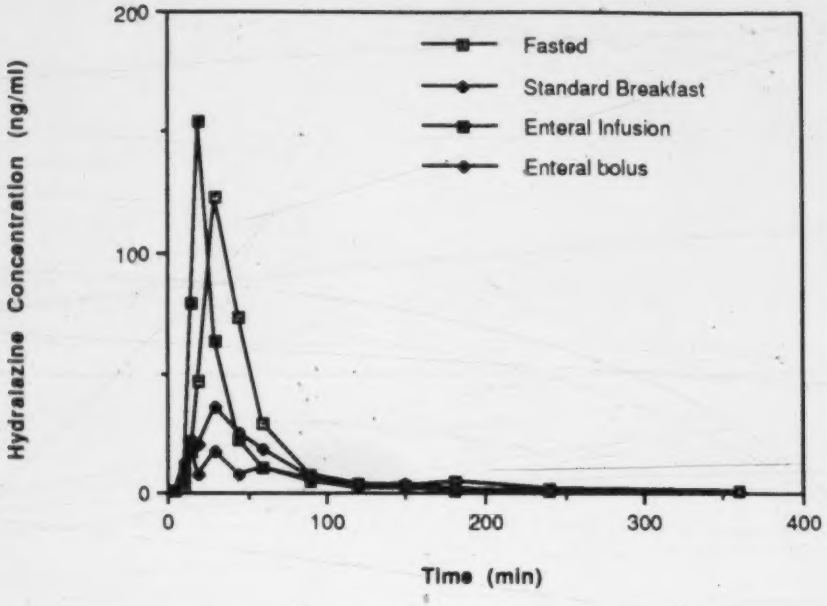


Figure 7-7: Hydralazine concentration *vs.* time profiles for Patient G (slow acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

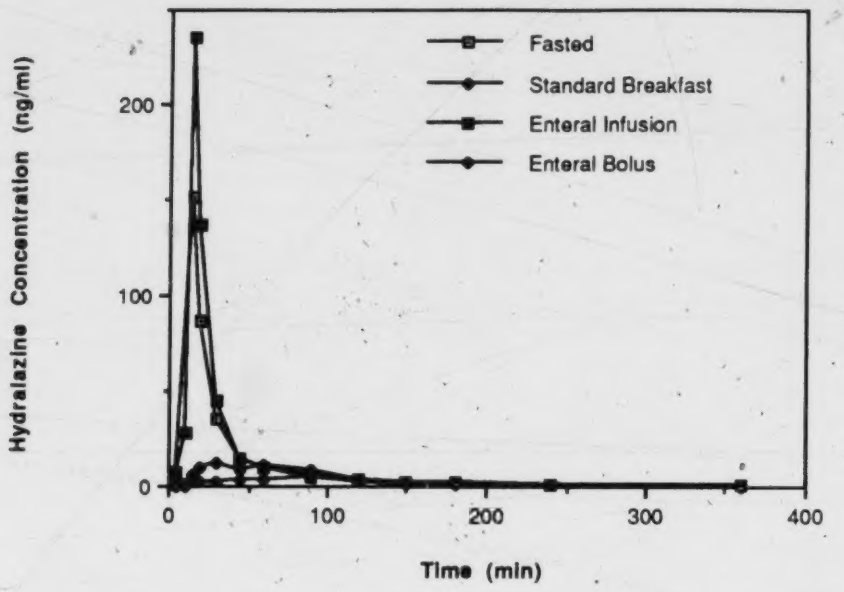


Figure 7-8: Concentration *vs.* time profiles for Patient H (slow acetylator) after a 50 mg oral dose following an overnight fast, with a standard breakfast, during an enteral infusion, or with a bolus of enteral nutrients.

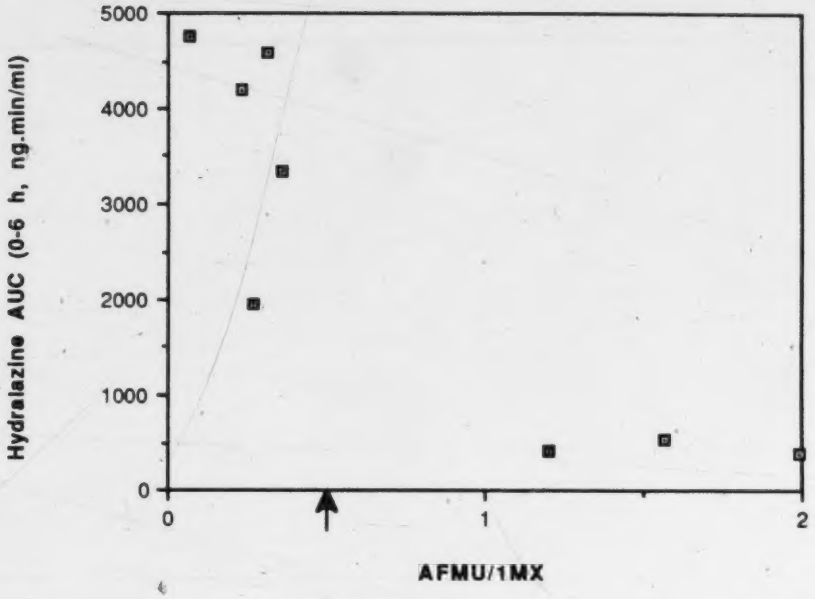


Figure 7-9. Hydralazine $AUC_{0\text{-}6\,h}$, fasted condition *vs.* molar ratio of the caffeine metabolites AFMU to 1MX. The arrow denotes the cutoff between slow (<0.5) and fast (>0.5) acetylators.

## 7.7 References


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# 8. General Discussion and Conclusions

## 8.1 Discussion

The first objective of this project was to duplicate or develop an assay method for hydralazine. The approach published by Ludden *et al.* (1) offered the most advantages, but in our hands, decomposition of the derivatives under the published conditions made the assay unsuitable. The publication of the work in Chapter 2 led Ludden *et al.* to publish stability data for their assay, which were acceptable (2), but the derivatives were only shown to be stable when EDTA was used as the anticoagulant and only when the assay was carried out in human blood. This method was found to be unsuitable for use in canine or porcine blood (Chapter 2, Unpublished data). Iwaki *et al.* (3) have used the method to assay hydralazine in rat blood.

The use of p-nitrobenzaldehyde rather than p-anisaldehyde as the derivatizing reagent solved the stability problems which hampered the previously published method without introducing new complications, and the sensitivity was improved. The resulting assay for hydralazine is quite robust. It is also very easy to duplicate. New, inexperienced personnel learned the procedures quickly and in less than 2 weeks could carry out the entire method independently.

The second objective was to evaluate the dog as an animal model. The parent drug kinetics of hydralazine were shown to be similar between dogs and slow acetylating humans, with both species exhibiting high total body clearance, a large steady state volume of distribution and saturation of metabolism. In addition, food was shown to cause a decrease in bioavailability, in agreement with the observations of Shepherd *et al.* (4 - 6) in the human. The findings of Shepherd *et al.* (4 - 6) were

confirmed in humans using the new assay method in the study described in Chapter 7. The dog, therefore, appears to be a useful animal model.

The secondary peaks seen in the oral data from Chapter 5 were repeated less often in the study in Chapter 6. When they did occur in the peripheral (jugular) blood profiles, they were not accompanied by concomitant secondary peaks in the other vessels. Their cause remains unknown, but it is not likely to involve absorption or enterohepatic recycling.

Propranolol and hydralazine share some important pharmacokinetic features. Both are subject to extensive first pass metabolism after oral dosage, both can exhibit saturation of metabolism within the therapeutic concentration range, and food has been claimed to increase the bioavailability of both drugs. Hydralazine also interacts with propranolol to increase its bioavailability in a similar manner to food. It was reasoned from this that the two drugs could share some hepatic mechanisms of the food effect. Information about the effect of saturation kinetics on the predicted mechanisms of this interaction was lacking, however. Since the food effect has been more extensively studied in propranolol than in any other high first pass drug, and pooled Michaelis-Menten parameters were available in the literature, it was selected as the best model drug for the simulation of the effect of hepatic enzyme saturation on the food interaction.

The conclusions, that saturation of metabolism may reduce the expected contribution of hepatic blood flow to propranolol bioavailability changes, and that some mechanism involving reduction of hepatic metabolism is likely, point to the necessity of having some measure of hepatic metabolic activity in the hydralazine studies. The discovery that hydralazine $Cl_{int}$ is increased with food was opposite to what was predicted, but the propranolol simulations helped to focus attention on the plausibility of a reduction of plasma protein binding being involved in the food effect on

hydralazine elimination. It was concluded that the first pass metabolism of propranolol is likely reduced mainly through nutrient-mediated increase of $K_m$ and/or reduction of $V_{max}$ to cause the observed bioavailability increase after food. This mechanism cannot be ruled out with hydralazine. If the major mechanism causing an increase in intrinsic clearance is a large food-related reduction of plasma protein binding, then this could mask changes in hepatic enzyme activity. While further investigation of this is warranted, the chances of successfully quantifying the contribution of plasma protein binding changes to the food interaction with hydralazine are slight, with presently available technology.

The development of the dog as a chronic, conscious animal model in which the physiological model parameters could be measured presented a considerable technical challenge. Somewhat less control over the experimental situation was possible, with the result that the data were more variable than would be expected from studies on anesthetized animals or isolated organs. Nevertheless, useful information that could be gathered in no other manner was made available. It was discovered that food causes concomitant increases in hepatic blood flow and intrinsic clearance of hydralazine. Hepatic bioavailability was changed little while hepatic clearance was increased. The main mechanism of the food interaction appears to be an increase in the systemic clearance of hydralazine. The systemic and hepatic mechanisms together contribute to a large reduction of hydralazine bioavailability with co-administration of food. The effect of saturation of metabolism could not be measured for two reasons. First, maximum influx of drug coincided with distribution and equilibration processes, and these could not be separated from possible saturation phenomena. Secondly, the drug dose may not have been sufficiently high to cause saturation of metabolism in all subjects. Further study will be required in this area. Experimental designs incorporating steady state

infusions would be most useful for the estimation of Michaelis-Menten parameters, but in the conscious animal, infusions producing saturation of metabolism could be toxic.

Unexpectedly, evidence of pregastric absorption of drug was uncovered. While this does not affect the validity of the hepatic data, it could lead to an overestimation of the oral bioavailability when it is calculated from peripheral blood drug concentrations. Variability of pharmacokinetic data secondary to irreproducibility of tablet dissolution profiles has led to the use of drug solutions for oral dosing. In the human study described in Chapter 7, the patients were given oral solutions of hydralazine for this reason. Pregastric absorption could, however, lead to undetectable errors in the estimation of pharmacokinetic parameters when this is done. Ludden *et al.* (2) recorded similar AUC values after oral solutions or immediate release tablets of hydralazine were administered to human subjects, but this does not necessarily validate the use of oral solutions. The simulations show that with only peripheral blood concentrations available, pregastric absorption of a large portion of the dose could be impossible to distinguish from gastrointestinal absorption of the entire dose. In support of the use of hydralazine solutions in the humans studies, differences in protocol from the animal studies should be pointed out. The human subjects were much more cooperative about taking their doses of hydralazine than were the canine subjects. The doses were administered quickly, and because of the bitter taste of the drug, the humans never failed to quickly drink the 100 ml of water which followed. The dogs, on the other hand, often held the diluted drug solution in their mouths for a period before swallowing, and the dose was administered in repeated small portions. Because the drug was diluted into 50 ml, and also because the time required for administration could lead to problems with the first sample, additional water was not given to the dogs after all the drug solution was swallowed. Therefore, the contact time between drug and oral mucosal membranes was perhaps longer in the dog experiments, possibly leading to a

greater amount of pregastric absorption than in the human experiments. There is no way to quantify this difference, however. Nevertheless, it must be recommended that in future clinical pharmacokinetic studies conducted on orally administered hydralazine, only tablet dosage forms should be used.

This finding presents a reason to investigate buccal and esophageal absorption of drugs before using liquid dosage forms when these are not administered in the clinical situation. Specifically for hydralazine, pregastric absorption could present a drug development opportunity whereby the toxic implications and reduced bioavailability associated with first pass metabolism could be avoided. This is another field requiring additional investigation.

The last study (Chapter 7), using the new assay, confirmed that food causes reduced bioavailability of hydralazine in humans, similarly to the observation in the dog and confirming the findings of Shepherd *et al.* (4 - 6). In addition, the data from the different nutrient formulations indicated that the rate of nutrient administration was important in determining whether the food effect would occur, and that the physical form of the nutrients did not significantly affect the change in bioavailability. This allows the investigation of the contribution of different nutrients to the food interaction using purified ingredients. The study also demonstrates the clinical importance of accounting for possible food interactions in patients receiving enteral nutrition and drugs together. Further study of this topic is necessary.

It would be difficult to explain the discrepancy between the data presented here or those of Shepherd *et al.* (4 - 6) and the studies by Melander *et al.* (7) and Liedholm *et al.* (8), except by the differences in assay specificity proposed by Shepherd *et al.* (4). Other differences between the studies do exist, however. One difference which could be important is that in the study described in Chapter 7 and that of Shepherd *et al.* (4), a liquid dosage form was administered rather than the regular release tablets used in the

other studies. The $t_{max}$ values differ considerably between the two dosage forms. It would be tempting to explain the discrepancy on the basis of absorption rate, because the relative absorption time and duration of the food effect could have a significant impact on the changes in bioavailability (Chapter 4). In two further studies by Shepherd *et al.* (5, 6), however, the tablet dosage form was incorporated into the designs, and hydralazine bioavailability was significantly reduced by food in both experiments.

The observations in the dog could provide an explanation of the underlying mechanisms of the food effect in the human. The theory proposed by McLean *et al.* (12) predicted that a transient increase in hepatic blood flow, such as was seen in both the human and the dog, would lead to an increase in bioavailability. In the dog, however, the increase in flow was accompanied by an increase in hepatic intrinsic clearance. The sum of the two processes led to a slight increase in hepatic extraction, so bioavailability was actually slightly reduced. The significant reduction in AUC after food is due to increased hepatic and systemic clearance rather than large changes in bioavailability. Although the food effect appears to last longer in the dog than in the human (13, 14), most of the drug has been cleared by both species after a short period (about 2 h).

## 8.2 Conclusions

1. It was shown that food reduces the bioavailability of orally administered hydralazine, in both the human and in the dog.
2. The simulations indicated that the predictions by physiological models would change at least for propranolol when linear terms are replaced by Michaelis-Menten parameters. The predicted effect of hepatic blood flow changes would be decreased and the effect of $K_m$ and/or $V_{max}$ changes would be dominant.
3. The dog was shown to be a useful animal model for the study of the interaction between food and hydralazine.
4. It was demonstrated that food causes an increase in hepatic intrinsic clearance as well as an increase in portal venous blood flow. The resultant changes to hepatic extraction were small, but an increase in hepatic clearance was shown. It was not possible to determine the individual contributions of changes to $K_m$, $V_{max}$ and $f_b$ values to the increase in intrinsic clearance.
5. It was determined that changes in hepatic parameters were not necessarily the major determinants of bioavailability changes caused by food in all subjects, as was hypothesized in the introduction. Rather, the change in AUC was associated more with an increase in systemic clearance. In addition, possible pregastric absorption, which could not be quantified, interfered with the determination of absorption changes.

In conclusion, this project has contributed to the elucidation of the mechanism of the food interaction with hydralazine, but has also uncovered many questions worth pursuing in future work. A chronic conscious dog model has been developed, which will be valuable for the study of other drug interactions involving the liver.

## 8.3 References


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## 9. Appendix: Simulation Data for Chapter 6

A model for hydralazine was designed using the computer program "STELLA " for the Macintosh (High Performance Systems, Lyme, NH). A schematic diagram of the model is shown in Figure 6-9.

### 9.1 Assumptions

1. The kinetic parameters derived for the group of dogs in Chapter 5 are valid for the present group. This is reasonable, given the agreement between the fasted AUC values for both groups. This assumption is required so that intravenous data from the previous study can be used to determine systemic parameters.
2. Systemic elimination of hydralazine by pyruvate hydrazone formation takes place at the same rate *in vivo* as *in vitro*, and can occur throughout the total body water. Both substrates are distributed into the cells as well as extracellularly, and the reaction occurs without catalysis by enzymes.
3. The changes in hepatic blood flow after an intravenous dose are the same as after an oral dose. This is a compromise in the absence of flow data after an iv hydralazine dose. Because hydralazine is absorbed quickly, the response should be similar to that after an iv dose.
4. All processes are governed by linear kinetics. Evidence for saturation of hepatic metabolism was encountered in only two of twelve cases.
5. All intercompartmental rate constants are first order.
6. The distribution of hydralazine in the dog occurs in at least 3 phases, corresponding to the total body water, in which systemic metabolism takes place, a quickly equilibrating phase, corresponding to the volume into which the drug is partitioned and a slowly equilibrating deep tissue compartment where

drug can accumulate, corresponding to the tissue binding sites observed in the radiolabelling studies (see Chapter 1).

7. All compartments are well mixed, including the liver. The "well stirred" model was used because it is mathematically simple. The more complex "parallel tube" model may have described the initial sample concentrations better (giving lower HV concentrations on the first sample than the WS model), but this feature could also be accounted for by an absorption lag time between the pregastric and gastrointestinal dose reservoirs. Because the extraction ratio is moderate, differences between the predictions of the models are insignificant (4), and both models should give qualitatively similar results.

## 9.2 Equations

The model was constructed according to Figure 6-9. The equations describing the flow of drug through the model are as follows:

Rates of change of the amount of drug in the various compartments,

$$dX_{VC}/dt = HV + K_{tvc} * X_T + K_{dvc} * X_D + K_{pga} * X_{PG} - PV - HA - K_{vct} * X_{VC} - K_{vcd} * X_{VC} - K_{ivm} * X_{VC}$$

$$dX_T/dt = K_{vct} * X_{VC} - K_{tvc} * X_T$$

$$dX_D/dt = K_{vcd} * X_{VC} - K_{dvc} * X_D$$

$$dX_{GIDR}/dt = -K_{gid} * X_{GIDR}$$

$$dX_{GI}/dt = K_{gid} * X_{GIDR} - K_{gia} * X_{GI}$$

$$dX_{PGDR}/dt = -K_{pgd} * X_{PGDR}$$

$$dX_{PG}/dt = K_{pgd} * X_{PGDR} - K_{pga} * X_{PG}$$

$$dX_L/dt = PV + HA - HV - R$$

$$PV = X_{GI} * K_{gia} + (X_{VC} / V_{VC}) * Q_{pv}$$

$$HA = (X_{VC} / V_{VC}) * Q_{ha}$$

$HV = (X_{liver} / V_{liver}) * Q_h$

$Input = HA + PV$

$E = Cl_{int} / (Cl_{int} + Q_h)$ (4, WS model)

$R = Input * E = (HA + PV) * Cl_{int} / (Cl_{int} + Q_h)$

$AUC = \int_0^{\infty} X_{VC}/V_{VC}\, dt$

Where:

$X_{VC}$ = The amount of drug in the Vascular Compartment.

$X_T$ = The amount of drug in the Tissue Compartment.

$X_D$ = The amount of drug in the Deep Tissue Compartment.

$X_L$ = The amount of drug in the Liver Compartment.

$X_{GIDR}$ = The amount cf drug in the Gastrointestinal Dose Reservoir Compartment.

$X_{GI}$ = The amount of drug in the Gastrointestinal Compartment.

$X_{PGDR}$ = The amount of drug in the Pregastric Dose Reservoir Compartment.

$X_{PG}$ = The amount of drug in the Pregastric Compartment.

The K values are the first order intercompartmental rate constants. The absorption rate constants were adjusted to give $t_{max}$ values close to observed values.

PV, HA and HV are the fluxes associated with the Portal vein, Hepatic artery and Hepatic vein, respectively.

R = The rate of hepatic metabolism.

The Q values are the blood flow rates in the subscripted vessels.

Clint = Hepatic intrinsic clearance.

AUC = Area under the concentration *vs.* time curve

The constants were given the following values:

$K_{tvc} = 0.1\ min^{-1}$

$K_{vct} = 0.1\ min^{-1}$

$K_{dvc} = 0.005\ min^{-1}$

$K_{vcd} = 0.01\ min^{-1}$

$K_{ivm} = 0.063\ min^{-1}$ (reflecting the in vitro half life of hydralazine in the dog, 11 min)

$K_{gia} = 0.22\ min^{-1}$

$K_{gid} = 0.22\ min^{-1}$

$K_{pga} = 0.1\ min^{-1}$

$K_{pgd} = 0.2\ min^{-1}$

$V_{VC}$ = 1000 ml/kg, to approximate the distribution of the systemic co-substrate of hydralazine, pyruvic acid.

$V_{liver}$ =100 ml/kg, in accordance with the large volume of distribution of hydralazine.

$Cl_{int}$ = 25 ml/min/kg

$Q_{pv}$ and $Q_{ha}$ were taken from the measured flow profiles and scaled to per kg values. Interpolated values were used between data points.

$Q_h = Q_{pv} + Q_{ha}$

To simulate an intravenous dose, $X_{VC}$ was set at 2.5 mg/kg and $X_{GIDR}$, $X_{GI}$, $X_{PGDR}$ and $X_{PG}$ were set at 0.

Using the parameters estimated from the intravenous dose, two oral doses with absorption only through the gastrointestinal route and two oral doses with absorption through both the gastrointestinal and pregastric routes were simulated. Simulated concentrations in the vascular compartment, portal vein and hepatic vein from all of these simulations were plotted against the measured data from the six subjects for comparison (Figures 6-10 to 6-12). The doses used were: 0.7 and 2.5 mg/kg through the gastrointestinal route, corresponding to the absorbed amount and to the entire dose

given, respectively; 0.7 mg/kg by the gastrointestinal route plus 1.8 mg/kg through pregastric absorption to account for the entire 2.5 mg/kg dose; and 0.7 mg/kg through the gastrointestinal route plus 1.4 mg/kg through pregastric absorption, corresponding to the absorbed amount plus enough to make 84% of the dose, approximately what was measured by Wagner (6-16) using radiolabelled hydralazine in dogs.